

Redox-Related Mechanisms to Rebalance Cancer-Deregulated Cell Growth

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1 **Redox-related mechanisms to rebalance cancer-deregulated cell growth**

2 **The anticarcinogenic mechanisms of regeneration**

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35 **Abstract**

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37 A delicate balance exists between the process of carcinogenesis and tissue regeneration. A number of malignant
38 tumours are considered the outcome of an impaired or incomplete regeneration process, resulting in persistently
39 dividing cells. Regeneration-competent tissues and animals are able to prevent and counteract growth
40 abnormalities and seem to have a low vulnerability to chemical carcinogenesis. Cancer cell survival depends,
41 among other things, on various redox-related mechanisms, which are targets of currently developed therapies.
42 Disadvantages of these therapies are a lack of specificity and drug resistance. As the majority of these redox-
43 related mechanisms also play an important role in successful and coordinated cell functioning and reproduction,
44 the regeneration process offers a unique parallel context for modern cancer research. This review focuses on the
45 interconnections between regeneration and carcinogenesis and how an understanding of regenerative forces and
46 redox-controlled mechanisms could contribute to the identification of new therapeutic targets to block the growth
47 and survival of cancer cells.

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49 **Keywords:** Carcinogenesis; Regeneration; Stem cells; Redox-related mechanisms; Anticarcinogenic therapies.

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69 **1. Reactive oxygen species**

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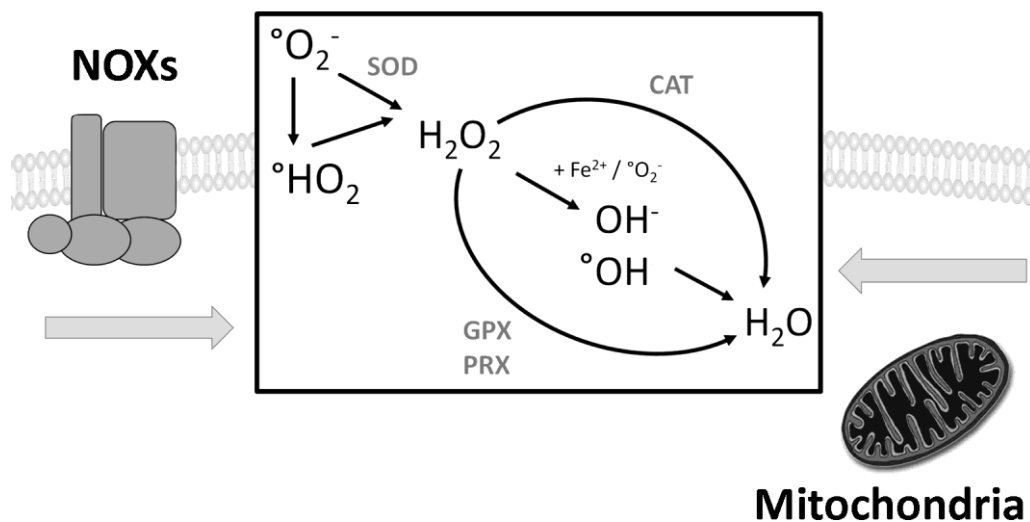
71 **1.1. Different types of reactive oxygen species**

72 Reactive oxygen species or ROS are reactive molecules that originate from oxygen as by-products of aerobic
73 metabolism or through enzymatic production [1-4] (Fig. 1). Oxygen (O₂) in its ground state contains two unpaired
74 electrons with parallel spins (triplet oxygen) and is not that reactive. The excited state of molecular oxygen is
75 singlet oxygen, which is formed via photo-excitation. Singlet oxygen has two electrons with antiparallel spins and
76 forms covalent bonds with other molecules more easily [4, 5]. The superoxide anion radical (^oO₂⁻) is formed after
77 a one-electron reduction of oxygen [3, 6]. This radical functions both as a reductant as well as an oxidant, is short-
78 lived and is not likely to diffuse across the cell membranes, which makes its effects rather localized [6]. When
79 superoxide receives a proton the more reactive hydroperoxyl radical (^oHO₂) is generated. The two-electron
80 reduction product of oxygen is hydrogen peroxide (H₂O₂), one of the most stable ROS. Given that it easily crosses
81 membranes, an important signalling function is attributed to this molecule [6]. The Haber-Weiss reaction involves
82 the so-called Fenton reaction in which hydrogen peroxide reacts with ferrous ions (Fe²⁺), yielding ferric ions (Fe³⁺),
83 a hydroxyl radical (^oOH) and a hydroxide anion (OH⁻). The nitric oxide radical (^oNO) plays an important role in
84 signal transduction and metabolism of nitric oxide gives rise to a series of compounds called reactive nitrogen
85 species (RNS) [1, 3]. Nitric oxide is formed in higher organisms through the oxidation of a terminal guanido-
86 nitrogen atom of L-arginine, which is mostly catalysed by the enzyme nitric oxide synthase (NOS) [3]. When the
87 superoxide anion reacts with nitric oxide, the highly reactive peroxynitrite (ONOO⁻) is formed [6]. Other RNS
88 include the nitrosonium cation (NO⁺) and the nitroxyl anion (NO⁻) [3].

89 **1.2. Physiological sources of ROS production**

90 Although most ROS are produced as by-products of metabolic and enzymatic activities, aerobic organisms also
91 utilize and actively produce ROS for numerous important physiological processes [6, 7]. Oxidoreductases catalyze
92 the electron transfer from the reductant (electron donor) to the oxidant (electron acceptor) in aerobic conditions
93 leading to the production of ROS. Multiple oxidoreductases are localised in different cellular compartments and
94 have been identified so far as potential ROS sources, including cyclooxygenase (luminal side of endoplasmic
95 reticulum and nuclear membrane), lipoxygenase (cytosol, nucleus), cytochrome P450 enzymes (endoplasmic
96 reticulum, mitochondria), nitric oxide synthase (different subcellular compartments, including the sarcoplasmic
97 reticulum, mitochondria and peroxisome), xanthine oxidase (cytosol, mitochondrial matrix, peroxisome),
98 mitochondrial NADH/ubiquinone oxidoreductase (complex I) and NADPH oxidase (NOX, cell membrane) [8].
99 Despite the diversity of all these ROS-producing enzymes, the majority of cellular ROS is produced by the
100 mitochondrial respiratory complexes and the NADPH oxidases.

101



102

103 **Figure 1: Production of reactive oxygen species.** The two main physiological sources of ROS production are the
 104 transmembrane NADPH oxidase (NOX) enzymes and the mitochondrial complexes I and III. A simplified
 105 overview of the different ROS species and the involved enzymes is presented. Superoxide anions ($^{\circ}\text{O}_2^-$) give rise
 106 to hydroperoxyl radicals ($^{\circ}\text{HO}_2$) or hydrogen peroxide (H_2O_2), through superoxide dismutase (SOD) activity. The
 107 reaction of hydrogen peroxide (H_2O_2) with Fe^{2+} in the Fenton reaction leads to the formation of the hydroxyl
 108 radical ($^{\circ}\text{OH}$) and hydroxide anion (OH^-), which can eventually be converted to molecular water (H_2O). Enzymatic
 109 activity of catalase (CAT), glutathione peroxidase (GPX) and peroxiredoxin (PRX) also convert H_2O_2 to molecular
 110 water.

111 *1.2.1. Mitochondrial respiratory complexes*

112 Under normal physiological conditions, most of the ROS present in cells (discussed in section 1.1) are produced
 113 indirectly as by-products of the mitochondrial respiratory chain. Oxygen acts as the final acceptor of the electrons
 114 transferred in the mitochondrial chain, in the end undergoing a four-electron reduction to form water. The
 115 generation of the superoxide anion, via electron leakage, occurs primarily at complex I (NADH/ubiquinone
 116 oxidoreductase) and complex III (ubiquinol/cytochrome c oxidoreductase) of the mitochondrial chain, depending
 117 on the metabolic state of the mitochondria [9, 10]. When both respiration and ATP production are low, the
 118 production of superoxide anions is high and the reverse electron transfer (RET) and FMN (flavin mononucleotide,
 119 located in complex I) are the primary sources of superoxide anion production. When respiration and ATP
 120 production are high, the production of superoxide anions is considerably lowered [10]. Approximately 0.12-2% of
 121 the respiratory oxygen results in the superoxide anion *in vitro*, as measured in isolated mitochondria. However,
 122 the actual *in vivo* values of mitochondrial ROS production are most likely considerably lower, probably because
 123 the isolated mitochondria are maintained in high non-physiological concentrations of oxygen [2]. The produced
 124 superoxide anions are converted into hydrogen peroxide by manganese superoxide dismutase (MnSOD), the only
 125 isoform of superoxide dismutase present in the mitochondrial matrix [10]. In contrast to the superoxide anions,
 126 hydrogen peroxide is able to penetrate the mitochondrial membranes, thereby affecting cell signalling events.

127 *1.2.2. NADPH oxidases*

128 NADPH oxidases or NOX enzymes distinguish themselves from other oxidoreductases, including the
129 mitochondrial complexes, in that they produce ROS as an end product instead of creating them as by-products of
130 their catalytic pathways. So far, five human NOX isoforms (NOX 1-5) have been identified as well as two dual
131 oxidases (DUOX 1 and 2). All NOXs are comprised of membrane-bound subunits and they all differ from one
132 another in terms of tissue distribution, domain structure, subunit requirements and mechanisms of activation. The
133 catalytic subunit of all NOX isoforms consists of a C-terminal dehydrogenase domain with an NADPH binding
134 site together with a bound flavin adenine dinucleotide (FAD). When the NOX enzymes are activated and their
135 subunits have assembled, the electrons of NADPH are first transferred to FAD and subsequently to the two haem
136 groups bound to the N-terminal domain, which finally passes them to two molecular oxygen molecules across the
137 membrane forming superoxide anions [6]. NOX-derived superoxide is generated in the extracellular environment
138 and dismutated quickly (catalysed by CuZnSOD) to hydrogen peroxide. Hydrogen peroxide diffuses freely across
139 cell membranes, most likely facilitated by the presence of aquaporin channels [11].

140 Genes encoding NOX subunits are found in animals as well as plants, but not in prokaryotic organisms. Their early
141 evolutionary appearance and conservation in both lower and higher eukaryotes as well as their wide distribution
142 in various cell types (especially in mammals) are indicative of the fundamental roles that NOXs probably play in
143 cell functioning. Several studies have shown their importance in the modulation of redox-sensitive, intracellular
144 signalling pathways, including the activation of certain transcription factors (*e.g.* AP-1 and NF- κ B) [8], as well as
145 in physiological processes such as immune responses and development. In order to control the redox balance, cells
146 possess various direct and indirect antioxidant mechanisms to regulate the ROS levels.

147 **1.3. Redox regulation and signalling**

148 Despite their positive signalling function, too much ROS can induce irreversible oxidative damage to
149 macromolecules such as nucleic acids, lipids and proteins, resulting in the loss of cellular function or cell death.
150 This redox-induced damage has been linked to the progression of numerous pathologies such as cardiovascular
151 diseases, inflammation, neurological malfunctioning and cancer [12]. In order to maintain homeostasis, the ROS
152 metabolism is tightly regulated by a variety of redox mechanisms that control the redox (oxidant/antioxidant)
153 balance [13].

154 The antioxidative system is composed of nonenzymatic and enzymatic antioxidants. Nonenzymatic antioxidants
155 are classified as metabolic antioxidants (*e.g.*, glutathione, L-arginine, metallothioneins and ferritins) and dietary
156 antioxidants (*e.g.* tocopherol, ascorbate and β -carotene) [14]. Some act as chain-breaking antioxidants (by
157 intercepting the chain-carrying radicals), thereby slowing down or stopping oxidative chain reactions (*e.g.*
158 tocopherol intercepts peroxy radicals). Others act as preventive antioxidants by intercepting oxidizing species
159 before damage occurs (*e.g.* β -carotene quenches singlet oxygen or metallothionein chelates toxic elements such as
160 Cd, As, Hg, *etc.*) [13]. Enzymatic antioxidants are considered preventive as they catalyse neutralization of ROS
161 by elimination [15]. The enzyme superoxide dismutase (SOD) catalyses the dismutation of superoxide into oxygen
162 and hydrogen peroxide. In human cells, there are three isoforms of SOD differing in their subcellular location and
163 metallic cofactor, *i.e.* cytosolic CuZnSOD (SOD1), mitochondrial MnSOD (SOD2) and extracellular CuZnSOD
164 (SOD3). The enzyme catalase (CAT) decomposes hydrogen peroxide to oxygen and water. Glutathione
165 peroxidases (GPX) reduce hydrogen peroxide to water, while both non-lipid and lipid hydroperoxides are reduced

166 to alcohols and water, thereby oxidizing the antioxidant metabolite glutathione (GSH). The oxidized glutathione
167 cofactor (GS-SG; glutathione disulphide) is then reduced back to its original state (2 GSH) by glutathione
168 reductase (GR). Peroxiredoxins (PRX) catalyse the reduction of hydrogen peroxide as well as organic
169 hydroperoxides such as cytokine-induced peroxide. Thioredoxins (TRXs), localized in the cytoplasm and
170 mitochondria, consist of multiple isoforms allowing the reduction of oxidized proteins by cysteine thiol-disulfide
171 exchange [15]. All these nonenzymatic and enzymatic antioxidant systems maintain a controlled redox balance
172 and protect the cells against oxidative stress.

173 The enzymatic antioxidant defence system is tightly regulated by various redox-controlled transcription factors,
174 such as the nuclear factor erythroid-2-related factor 2 (Nrf2), the endonuclease 1/redox effector factor 1
175 (APE1/Ref-1), the family of forkhead box type O (FoxO), the ataxia-telangiectasia mutated (ATM) protein kinase
176 and the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). Nrf2 activates the transcription of
177 several antioxidant enzymes such as SOD1, CAT, GPX2, GR and TRX via the antioxidant responsive element
178 (ARE). In physiological conditions, Nrf2 is retained in the cytoplasm by anchoring to the Kelch-like ECH-
179 associated protein 1 (Keap1). Internal or external induced elevation of ROS levels oxidize the reactive thiol groups
180 of Keap1 causing the dissociation of Nrf2. The activated Nrf2 translocates into the nucleus and forms a heterodimer
181 with small Maf proteins to activate genes in the oxidative stress response [4, 16-18]. APE1/Ref-1 plays a pivotal
182 role in the DNA base excision repair pathway and controls the intracellular redox state in two ways: by inhibiting
183 Rac-1 regulated NOX and by reducing oxidized transcription factors such as hypoxia-inducible factor 1α (HIF-
184 1α), Nrf2, and the tumour suppressor protein p53 [19, 20]. The latter transcription factor p53 is an essential
185 component in the regulation of cell cycle arrest, cellular senescence and apoptosis and also plays an antioxidant
186 role in the response to oxidative stress [1, 21]. FoxO transcription factors control intracellular ROS levels by
187 upregulating the antioxidative enzymes CAT and SOD2 [22]. ATM protein kinase, a regulator of the cellular
188 response to DNA double-stranded breaks (DSBs), controls the intracellular levels of ROS, among others, by
189 stimulating NADPH synthesis leading to suppression of protein synthesis and activation of autophagy [23-25].
190 NF-κB, a transcription factor playing a crucial role in cell survival, is activated by oxidative stress and in turn
191 activates genes coding for ferritin (FT) and SOD2 [26].

192 ROS not only activate the antioxidant defence system, they also regulate important processes such as cell
193 proliferation, differentiation and survival through oxidation of redox-sensitive protein kinases and protein
194 phosphatases, mainly at the cysteine residue. The cysteine sulfhydryl group undergoes different degrees of
195 oxidations resulting in the generation of sulphenic acid (-SOH), sulphinic acid (-SO₂H) or sulphonic acid (-SO₃H).
196 Cysteine oxidation either leads to inhibition or activation of the downstream proteins [27, 28]. An example of such
197 an indirect regulation of transcription factors is the redox-controlled stimulation of MAP kinases (MAPKs). Three
198 MAPK enzymes have been identified: extracellular signal-regulated kinase (ERK), C-Jun N-terminal kinase (JNK)
199 and p38 kinase [29-31]. Their actions alter the expression of multiple genes involved in cell proliferation,
200 differentiation and apoptosis, including, among others, *cyclinD1* and *cdk2*. ROS-induced stimulation of MAPKs
201 occurs via the inhibition of 1) a negative MAPK regulator MKP, 2) thioredoxin (TRX), 3) a negative regulator of
202 the apoptosis signal-regulating kinase (ASK1), or via the activation of 1) a proto-oncogene, 2) a non-receptor
203 tyrosine kinase (SRC), 3) a positive regulator of MAPKs. Other pathways that are activated in response to an
204 altered redox state are the phosphoinositide 3-kinase (PI3K)/Akt pathway, the NF-κB system, p53 activation, and

205 the heat shock response. In general, the heat shock response, ERK, PI3K/Akt and NF- κ B signalling pathways are
206 involved in the prosurvival response of the cell, whereas activation of p53, JNK and p38 are linked to apoptosis
207 [2]. The balance between prosurvival and apoptotic responses strongly depends on the intensity and frequency of
208 the oxidative stimulus [4].

209 **1.4. ROS-(de)regulated processes**

210 ROS are important in numerous physiological processes including immunology, wound healing and angiogenesis.
211 However, an imbalance between the production and detoxification of ROS has been linked with ageing and with
212 a broad range of diseases such as cancer, insulin resistance, diabetes mellitus, cardiovascular diseases,
213 atherosclerosis and neurodegenerative conditions [32]. Here, we will focus on redox-related processes within the
214 framework of potential anticarcinogenic targets, the scope of this review.

215 *1.4.1. Cellular responses*

216

217 *ROS & cell death*

218 ROS affect the process of cell death in both a direct and indirect manner. Due to their chemically reactive state,
219 ROS molecules interact directly with various cellular components, including the nucleotides of DNA. These
220 interactions can cause single- and double-stranded DNA breaks, purine, pyrimidine or deoxyribose modifications
221 and DNA cross-links, leading to mutations [4, 33, 34]. The main ROS that cause DNA damage are the hydroxyl
222 radical ($^{\circ}\text{OH}$), singlet oxygen ($^1\text{O}_2$) and one-electron oxidants [35]. Dependent on the severity of the damage and
223 the ability of the cell to adapt to or resist the stress and repair the cellular damage, different responses are possible.
224 Moderate damage activates the repair machinery whereafter cell proliferation continues. However, when the DNA
225 damage is irreparable, cell death is initiated [2, 36]. ROS-induced cell death involves both necrosis and apoptosis,
226 depending on the severity of the insult. An important feature in preventing cell death is autophagy, which is
227 activated by ROS and once activated reduces oxidative stress by degrading proteins and damaged mitochondria
228 (mitophagy), a primary source of intracellular ROS [37, 38]. Some DNA regions are more susceptible to ROS-
229 induced damage, especially regions rich in guanine bases since different ROS, including singlet oxygen and
230 hydroxyl radicals, convert guanine to 8-oxo-7,8 dihydroguanine resulting in single base DNA damage. Due to
231 their high guanine content, telomeres - the protective and repetitive TTAGGG sequence at the end of the
232 chromosomes - are highly sensitive to ROS. Moreover, in contrast to the majority of the genomic DNA, the repair
233 system of the telomeric region is inefficient in repairing single-stranded breaks. Accelerated degradation of these
234 protective sequences eventually results in destruction of the DNA at the end of the chromosome [39, 40].
235 Interestingly, overexpression of the telomerase reverse transcriptase (TERT), the catalytic subunit of the
236 telomerase enzyme that elongates the telomeres and is linked with immortalization and stem cell populations,
237 reduces intracellular ROS levels and cell death [41, 42].

238 Besides inducing cell death through DNA damage, ROS also act as upstream signalling molecules in the induction
239 of apoptosis [36]. Chen and colleagues showed that ROS production results in apoptosis of neuronal cells in a
240 concentration- and time-dependent manner through activation of the MAPK pathways [43]. In some cells, a
241 positive feedback loop via p53 helps to reach critical ROS levels that induce a successful apoptotic response [2].
242 Moreover, the expression of antiapoptotic factors, such as Bcl-2 and Bcl-x, is also associated with protection

243 against ROS. During tumour necrosis factor (TNF- α)-induced apoptosis, Bcl-2 prevents the accumulation of ROS
244 and thus the subsequent apoptotic events (mitochondrial membrane depolarization, Bax relocalization, cytochrome
245 c release, caspase activation and nuclear fragmentation). Similarly, Bax and Bcl-x are able to respectively increase
246 or decrease GSH levels, without interfering with other antioxidant enzymes such as SOD, CAT, and GPX/GR [9].

247 *ROS & cell proliferation and differentiation*

248 The most important MAPK group via which ROS induce cell proliferation is the ERK family. This MAPK pathway
249 is a known intracellular checkpoint of mitosis. Inhibiting or stimulating the ERK signalling results in either an
250 inhibition or enhancement of cell proliferation [29]. In this process, JNK acts as a final mediator of ERK to
251 stimulate cell proliferation. Cross-talk between the different MAPK cascades ultimately controls the balance
252 between proliferation, differentiation or apoptosis [29]. Considering their function, it is not surprising that ERK,
253 JNK and p38 MAPK signalling pathways are all associated with tumourigenesis. For example, in various cancer
254 types in both humans and mice, ERK1 and ERK2 are upregulated while JNK and p38 MAPK pathways are often
255 downregulated [31]. Another downstream mechanism through which ROS induce proliferation is the TRX/Ref-1
256 complex. Thioredoxins (TRXs) are small proteins containing a redox-active disulphide site, while Ref-1 is a
257 multifunctional protein with endonuclease and oxidoreductase activity. TRX reduces the oxidized Ref-1, which in
258 turn reduces the DNA-binding domain of various proliferation-inducing transcription factors, thereby enhancing
259 their activity [7].

260 *ROS & patterning*

261 Wnt proteins are essential for normal development. They not only regulate pathways controlling cellular processes
262 such as proliferation and differentiation, but also direct proper pattern formation. The canonical Wnt pathway acts
263 through β -catenin, which, upon accumulation in the cytoplasm, translocates to the nucleus and associates with
264 different transcription factors, hereby activating the expression of downstream target genes. In the absence of the
265 Wnt ligand, cytoplasmic β -catenin is degraded and the downstream transcription is inhibited. ROS, including
266 hydrogen peroxide, oxidize nucleoredoxin (NRX) which, in this oxidized state, inhibits the degradation of
267 cytoplasmic β -catenin. This results in the accumulation of β -catenin levels and the activation of downstream
268 transcription factors in the absence of the Wnt ligand [7, 36, 44-46]. The importance of ROS in Wnt/ β -catenin
269 signalling pathway was also demonstrated in *in vivo* experiments on larvae of *Xenopus* by Funato and co-workers.
270 They showed that overexpression of NRX inhibits the expression of early targets of Wnt-signalling whereas NRX
271 knockdown resulted in Wnt pathway activation with reduced expression of anterior markers and structures (*e.g.*
272 absence of eye development) [44]. In intestinal and colon epithelial cells, NOX1-generated ROS play an essential
273 role in the inactivation of NRX [46]. Moreover, a redox-dependent regulation of the Wnt pathway via NRX was
274 also observed in F9 teratocarcinoma cell lines [47, 48].

275 *ROS & stem cell maintenance*

276 Stem cells are characterized by their abilities to self-renew and to produce numerous differentiated progeny cells
277 [49]. The fate of stem cells is (co-)directed by the intra- and extracellular (stem cell niche) redox balance: low
278 ROS levels support stem cell maintenance and quiescence while slightly “higher” ROS levels induce stem cell
279 proliferation and eventually differentiation [1, 42, 50, 51]. A rise in ROS levels can induce apoptosis to prevent

280 the accumulation of damaged stem cells, as discussed previously [52]. On the other hand, if increased ROS levels
281 can be controlled, stem cell survival is achieved. This redox (re-)balancing is acquired either directly through the
282 activation of antioxidative systems or indirectly via signalling regulators such as PTEN, ATM, HIF, FoxO and
283 Nrf2. Tumour suppressor gene families, such as p53 and p38, are key mediators of ROS-regulated stem cell
284 renewal [51]. In planarians, which have an extensive population of pluripotent stem cells, expression of the tumour
285 suppressor gene p53 is largely restricted to the newly formed progeny of the stem cells and knocking down this
286 gene results in an increase in stem cell number and proliferation causing a depletion of the stem cell population
287 [49, 51, 53, 54].

288 Since a precise control of the redox balance is crucial for normal cellular functioning, it is not surprising that any
289 unwanted fluctuations in ROS levels can have devastating outcomes, including the formation of cancer. As ROS
290 are involved in both normal physiological processes as well as in diseases, we will focus on this dual role by
291 emphasizing their importance in two related processes: the physiological process of regeneration and the
292 pathological process cancer. The role of ROS in both the processes will be discussed in the following sections.

293 **2. ROS modulate carcinogenesis**

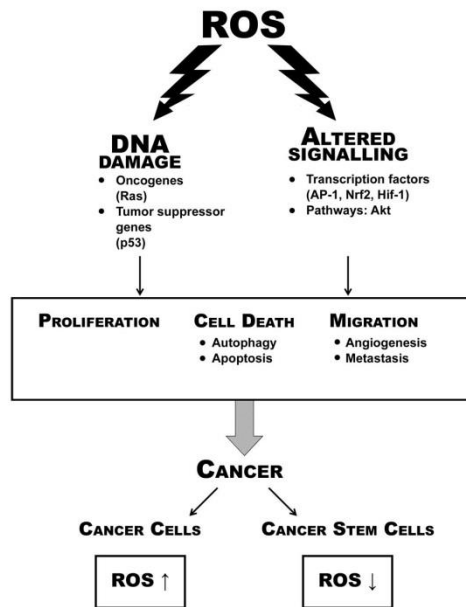
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295 **2.1. ROS as a core component of carcinogenesis**

296 Carcinogenesis is characterized by uncontrolled cell divisions, ultimately resulting in a malignant tumour which
297 invades tissues and metastasizes [55]. Classically, carcinogenesis consists of three stages: initiation, promotion
298 and progression [56].

299 DNA damaging effects of ROS are mainly involved in the initiation and progression stage (Fig. 2). Both exogenous
300 carcinogens as well as endogenic processes such as chronic inflammation increase oxidative stress and lead to
301 initiated cells [34]. The carcinogenic metal cadmium, for example, induces multiple cancers by increasing internal
302 oxidative damage [52]. It disturbs the redox balance via various mechanisms including the inhibition of antioxidant
303 enzymes (*e.g.* SOD), the depletion of ROS scavengers (*e.g.* GSH), the interference with the electron transport
304 chain causing superoxide anion production and the displacement of redox-active metals (*e.g.* Fe) leading to more
305 hydroxyl radicals. The hydroxyl radical ($^{\circ}\text{OH}$) induces 8-hydroxydeoxy guanosine (8-OHdG), one of the major
306 products of DNA oxidation. 8-hydroxydeoxy guanosine levels are elevated in various human cancers and animal
307 tumour models [33, 57, 58]. 8-OHdG not only pairs with cytosine but also with adenine residues, leading to a G:C
308 to T:A transversion, a mutation commonly found in proto-oncogenes, oncogenes and tumour suppressor genes
309 [33, 35, 57].

310



311

312 **Figure 2. Schematic overview of the roles of reactive oxygen species (ROS) in cancer.** ROS modulate the
 313 carcinogenic process by inducing DNA damage (*e.g.* in oncogenes such as *ras*) and by altering signalling pathways
 314 (*e.g.* via the regulation of transcription factors). In this way, ROS initiate and promote cancer through disturbing
 315 cell proliferation, death and migration. The amount of ROS necessary to drive carcinogenesis depends on the
 316 specific tumour cell type: while cancer cells have elevated ROS levels to accelerate DNA damage, cancer stem
 317 cells have lower intracellular ROS levels to facilitate the continuation of the tumour.

318 Proto-oncogenes mostly code for signal transduction components that promote proliferation, such as growth
 319 factors and receptors or transcription factors and, once mutated to an overactive oncogene, they induce
 320 uncontrolled proliferation. The *ras* oncogene - one of the first oncogenes identified - transfers extracellular signals
 321 to downstream signalling cascades such as MAPK pathways, eventually stimulating mitogenic processes.
 322 Normally, Ras activation has an intrinsic negative feedback loop to ensure a transient transmission of the signal
 323 but mutations in *ras* eliminate this regulating mechanism, leading to uncontrolled mitogenic activation [56, 59].
 324 These mutations are for example found in around 50% of colon cancers and up to 90% of pancreatic cancers [59].
 325 Tumour suppressor genes, on the other hand, regulate cell death programs such as apoptosis and, once
 326 downregulated or disturbed through mutations, support cell death resistance. The best-known tumour suppressor
 327 is undoubtedly p53, which functions in cell cycle arrest, DNA repair, senescence and apoptosis, and is one of the
 328 most frequently altered genes in cancer [60-63].

329 Apart from DNA damage, ROS also modulate the carcinogenic process via transcriptional and signalling
 330 alterations which underlie multiple mechanisms of cancer including the clonal expansion of initiated cells during
 331 the promotion phase. Commonly targeted transcription factors during carcinogenesis are AP-1, Nrf2 and HIF-1.
 332 AP-1 is activated via MAPK and JNK signalling pathways, induces growth and proliferation and reduces oxidized
 333 Nrf2 and HIF-1 α [33]. Nrf2 activates numerous stress-related genes and antioxidant enzymes in the presence of
 334 oxidative stress. This activation has been linked with aggressive behaviour of different types of cancer, such as

335 breast cancer, lung cancer, neuroblastoma and ovarian cancer [33, 64]. The activation of HIF-1 is triggered by a
336 reduced cellular oxygen content, which is often present in tumours as a consequence of their active metabolism
337 and continuous proliferation. Mitochondria function as O₂ sensors and signal hypoxia by releasing ROS to the
338 cytosol, which in turn stabilize HIF-1. HIF-1 has more than 60 target genes, all of which promote carcinogenesis
339 via various mechanisms such as cell survival, angiogenesis, invasion and metabolic reprogramming. Elevated HIF-
340 1 expression has been linked to poor outcome in various cancer types such as breast cancer [65], cervical cancer
341 [66] or pancreatic tumours [67]. Besides transcription factors, tyrosine kinases/phosphatases such as the kinase
342 Akt, which mediates cell survival via inhibiting proapoptotic transcription factors, are targets of ROS [68].

343 During the final steps of carcinogenesis, the progression stage, both the DNA damage and signalling defects caused
344 by the disturbed redox balance are important. In this way, ROS induce uncontrolled divisions and additional
345 mutations that drive the tumour towards a malignant neoplasm [33].

346 **2.2. ROS support the hallmarks of cancer**

347 Throughout the three phases of carcinogenesis, necessary "capabilities" for tumour growth and metastasis are
348 acquired, which are defined as "The hallmarks of cancer" by Hanahan and Weinberg [56]. ROS play a role in each
349 of these capabilities via mutagenic effects, alterations of signalling pathways, effects on the microenvironment and
350 on invasiveness (reviewed in [69] and [70]).

351 One of the most fundamental hallmarks of cancer is the ability to sustain proliferative signalling. Where normal
352 tissues meticulously regulate the amount of growth-enhancing signals to maintain homeostasis, cancer cells abuse
353 this system by continuously activating the cell cycle. ROS promote sustained proliferation on one hand by inducing
354 mutations of crucial cell cycle control genes such as proto-oncogenes, on the other hand by excessively activating
355 signalling pathways leading to proliferation [56, 59].

356 ROS also play a role in the avoidance of growth suppressor mechanisms, which is another hallmark of cancer. One
357 such mechanism is the suppression of proliferation by contact inhibition, which is believed to be important during
358 wound healing and regeneration, and for which Ras and Ras-induced ROS levels have to be low. Overactivating
359 mutations of *ras* elevate ROS levels and consequently result in a loss of contact inhibition [70]. Other cell-cycle-
360 related hallmarks in which ROS play a role are the resistance of apoptosis and the enabling of replicative
361 immortality. ROS-induced mutations can downregulate tumour suppressor genes involved in cell death programs
362 such as apoptosis and support cell death resistance, whereas unlimited replication capacities can be obtained by
363 ROS signalling increased telomerase expression [56, 60, 70, 71].

364 Angiogenesis is crucial to maintain continuously-active tumour cells by delivering nutrients and oxygen to the
365 growing cancer and removing waste products and carbon dioxide. In normal physiological processes (*e.g.* wound
366 healing) angiogenesis is only transiently active, but during tumour progression new vessels are continuously
367 growing. ROS directly promote angiogenesis by controlling the pro-angiogenic vascular endothelial growth factor
368 A (VEGF-A) and by activating the transcription factors NF- κ B, AP-1 and STAT1 (signal transducers and
369 activators of transcription family) [56, 69, 72, 73]. In doing so, ROS also support tumour invasion and metastasis
370 to a secondary niche. ROS play additional roles in invasion and metastasis via supporting Met (MET proto-
371 oncogene, receptor tyrosine kinase) overexpression and epithelial-mesenchymal transition (EMT), as well as

372 through the creation of invadopodia (which degrade the extracellular matrix) or the recruitment and transformation
373 of fibroblastic cells into carcinoma-associated myofibroblasts which secrete pro-invasive signals [56, 69, 70, 74,
374 75].

375 ROS also link tumour-promoting inflammation and cancer. Inflammatory cells produce ROS which attract more
376 inflammatory cells and lead to more ROS production [57]. Chronic inflammation is indeed strongly correlated
377 with cancer incidence [33, 57]. For example, colitis develops into colon cancer after inflammatory infiltration,
378 increased production of ROS and impairment of antioxidant defences resulting in genetic and epigenetic alterations
379 that promote cancer development [76]. On the other hand, exogenous-induced oxidative stress also leads to chronic
380 inflammation. For example, cigarette smoke produces pro-oxidants, causing inflammation of the bronchus and
381 eventually transformation of lung cells into malignant tumour cells [33, 57].

382 On top of their original six hallmarks, Hanahan & Weinberg (2011) recently added two emerging hallmarks:
383 deregulation of cellular energetics and avoidance of immune destruction. The deregulation of cellular energetics
384 results from the metabolic reprogramming of cancer cells or, as recently suggested, of adjacent stromal fibroblasts
385 [77-79]. This metabolic reprogramming, referred to as the Warburg effect, consists of an abnormal oxygen
386 metabolism with high rates of aerobic glycolysis leading to lactate production instead of mitochondrial oxidative
387 phosphorylation, even when ample oxygen is present [77-79]. In this process, cancer cells induce oxidative stress
388 in neighbouring stromal cells, which elicits their autophagic cell death via activation of NF- κ B and HIF-1 [80].
389 Autophagy in the stromal cells not only releases building blocks for cancer cells directly, it also reprograms the
390 stroma metabolically, hence leading to the Warburg effect [79-81]. Hereby, additional high-energy mitochondrial
391 fuels and biomass precursors (*e.g.* lactate, ketone bodies, fatty acids, and glutamine) are released to fuel the
392 anabolic cancer cells [79, 80]. Autophagy can thus work cytoprotective for growing tumours adapting to a stressful
393 environment. However, it should be noted that autophagy has a dual role and can on the other hand also prevent
394 carcinogenesis by removing damaged organelles such as mitochondria and, in this way reduce oxidative damage
395 and genomic instability [82]. The hallmark for which the involvement of ROS is still uncertain is the evasion of
396 cancer cells from immune destruction. It can be speculated that ROS activate the immune suppressor TGF- β
397 resulting in the inactivation of natural killer cells [83, 84]. However, since administration of TNF blockers, which
398 prevent TNF-induced ROS production, to patients with rheumatoid arthritis increased the risk for developing
399 lymphomas, the contribution of ROS in avoiding immune destruction is still uncertain [85].

400 **2.3. ROS regulation in cancer cells and cancer stem cells**

401

402 *2.3.1. ROS and cancer cells*

403 Neoplastic cells have altered redox systems and produce more ROS than normal cells [1, 86]. Chronically-elevated
404 levels of endogenous ROS in cancer cells accelerate DNA damage and alter signalling pathways, leading to
405 adaptive changes that play important roles in tumourigenesis, metastasis and drug resistance, and are correlated
406 with a bad prognosis [1, 87].

407 The mechanisms by which cancer cells increase ROS are diverse. They activate proto-oncogenes and oncogenes
408 that upregulate ROS-producing enzymes, inactivate antioxidants or stimulate the metabolism [1, 85, 88-91]. For
409 example, *ras*-transformed cells contain more superoxide via activation of NOX1 or inactivation of the antioxidant

410 sestrin, which is required for anchorage-independent growth, morphological transformation and tumorigenicity
411 [1, 70, 92, 93]. In addition, Ras itself is redox-regulated and activated by nitric oxide and, possibly, hydrogen
412 peroxide [70]. Loss or reductions of tumour suppressor genes also induce a redox-imbalance and elevated ROS
413 levels in many types of cancers. For example, a loss or decrease of *p53* disturbs its function in controlling genetic
414 stability and in preventing cancer by regulating the expression of antioxidant genes such as GPX1, MnSOD and
415 aldehyde dehydrogenase 4 (ALDH4) [1, 94]. In stromal fibroblasts, redox alterations resulting from a loss of *p53*
416 is a key inducer of epithelial transformation and cancer invasion via RNS-mediated ICAM1 signalling [95]. The
417 free radical production rate in cancer cells is also elevated by their increased glycolytic metabolism. Probably,
418 high rates of glycolysis and a metabolic shift towards the production of lactate reflect a less efficient ATP
419 production in the mitochondria and increases electron leakage from the respiratory chain [1, 81, 96-102]. The
420 reason why cancer cells use this aerobic glycolytic metabolism for energy requirements is still under debate. It
421 could be beneficial in the hypoxic tumour environment, created by extensive cell proliferation, unorganized
422 vasculature and irregular blood flow. Mitochondrial dysfunction as a consequence of mitochondrial DNA
423 mutations could also be responsible for respiratory inefficiency and could contribute to the Warburg effect [81,
424 96-101, 103].

425 While elevated ROS levels support tumour initiation, progression and promotion, they also increase the internal
426 stress level and make the tumour more vulnerable to cell death. Therefore, a dynamic ROS regulation in tumour
427 cells, which prevents cell death but suffices to stimulate cell survival and proliferation, is essential to induce
428 malignant transformation, metastasis and chemoresistance [1, 70, 86]. Most cancer cells adapt to persistently-
429 increased ROS levels by activating redox-sensitive transcription factors that upregulate ROS-scavenging enzymes
430 and systems such as SOD, GSS (glutathione synthetase), and GSH [1]. Oncogenes like *K-ras*, *B-raf* and *myc*
431 increase the resistance of cancer cells to oxidative stress by elevating the activity of the Nrf2 transcription factor,
432 which increases ROS detoxification and upregulates various cytoprotective genes [1, 70, 86]. Deletion of Nrf2
433 reduces the possibility of K-Ras to induce proliferation and tumorigenesis, which supports the importance of
434 ROS limitation in tumour outgrowth [86]. Human telomerase reverse transcriptases (hTERTs), the catalytic
435 subunits of telomerase holoenzymes, reduce basal cellular ROS levels and endogenous ROS production in cancer
436 cells, possibly through activation of NF- κ B, by increasing the ratio of reduced to oxidized glutathione, by
437 recovering oxidized peroxiredoxin to its nonoxidized form, and by elevating the activity of cytochrome c oxidase
438 [41]. Other strategies of cancer cells to avoid excessive oxidative damage include metastasis to escape ROS in the
439 primary tumour site, and the Warburg effect to increase oxidant-resistance [70].

440 If cancer cells fail to keep ROS levels below a certain cell-death threshold, ROS induce cancer cell death via
441 apoptosis, necrosis or autophagy, as has been reviewed by Gupta *et al.* [85]. The ability of cancer cells to
442 distinguish between ROS as a survival or apoptotic signal depends on the concentration, duration, type and site of
443 ROS production. For example, while modest ROS levels are required for the survival of cancer cells, excessive
444 levels cause senescence-induced tumour suppression or cell death and while NOX-derived ROS in response to
445 TNF α play a protective role, mitochondria-derived ROS promote apoptosis [1, 85, 86].

446 2.3.2. ROS and cancer stem cells

447 Cancer cells always keep a portion of ROS low cells with stem-like characteristics in the cell population, the cancer
448 stem cells. The hypothesis is that these tumour seeds are protected against DNA damage in order to facilitate the
449 continuation of the tumour [1]. Cancer stem cells, also called tumour-initiating cells or tumour-propagating cells,
450 are only a subset of the entire tumour population and share properties of self-renewal and chemo- and
451 radioresistance with normal stem cells. They have an increased capacity to initiate and sustain tumour growth,
452 which is important in the progression and the recurrence of malignant tumours [1, 100, 104-106]. Cancer stem
453 cells also play an important role in tumour angiogenesis by vascular endothelial growth factor (VEGF) signalling
454 [100]. Possibly, they are the source of all the tumour cells present in a malignant tumour [1, 100, 104-106].

455 Little is known about the redox balance of cancer stem cells. It is thought to resemble that of normal stem cells,
456 given the importance of this balance in stem cell maintenance, self-renewal and differentiation [1, 70, 100]. Just
457 as stem cells, cancer stem cells possess enhanced protection mechanisms against ROS-induced stress, which could
458 explain their survival and drug resistance. In comparison with non-stem cancer cells, they have lower intracellular
459 ROS levels due to increased expressions of free radical scavenging systems [1, 70, 104, 105]. For example,
460 expression of the (cancer) stem cell marker CD44, and especially the isoform CD44v, protects cancer stem cells
461 against high ROS levels in the tumour environment through stabilization of the glutamate-cysteine exchange
462 transporter and the consequent stimulation of intracellular GSH. The removal of CD44v results in increased ROS
463 levels and ROS-dependent p38 MAPK activation, making CD44v-targeted therapies that prevent ROS defence a
464 potential approach to kill cancer stem cells [1, 100, 106, 107]. The mechanisms of cancer stem cells to repair ROS-
465 induced damage are also thought to differ from non-stem cancer cells, with possibly enhanced activation of DNA
466 checkpoint kinases [1, 100]. Tumour dormancy, which temporarily halts metastatic growth and is induced by p38
467 MAPK, represents another protection mechanism of cancer stem cells against oxidative stress. In high-ROS
468 environments, dormant cancer stem cells survive injury and wait to expand the tumour in more favourable times
469 and safer conditions [70].

470 Moderate hypoxia in the tumour environment also becomes increasingly related to the cancer stem cell phenotype
471 and is thought to be beneficial for the survival, self-renewal and tumour growth of cancer stem cells [1, 100]. In
472 brain tumours, restricted oxygen conditions increase the cancer stem cell fraction and promote acquisition of a
473 stem-like status [100]. Cells under hypoxia display increased markers associated with stem-like phenotypes such
474 as CD44. It is hypothesized that the correlation of tumour hypoxia to poor patient outcome is related to an increase
475 in the proportion of cancer stem cells. As such, therapies that disrupt the microenvironmental conditions of hypoxia
476 may be critical in eliminating cancer stem cells [100].

477 In conclusion, we can state that it is crucial to determine molecular signalling pathways that maintain the redox
478 state in cancer cells and cancer stem cells. By abrogating the cell's survival mechanisms against redox imbalances,
479 the ability of redox-related therapies to halt cancer can be enlarged.

480 **3. ROS modulate the regeneration process**

481 In the previous section, we summarized the involvement of ROS in tumour initiation, growth and metastasis. To
482 highlight the dual role of ROS, we will also discuss their function in a normal physiological situation, *i.e.*
483 regeneration. Regeneration and cancer share similar underlying events, but result in a different outcome (section

484 3.1). As such, we will emphasize the redox-related aspects of the shared processes, among which cell proliferation,
485 apoptosis and differentiation.

486 Regeneration is a complex concept, defined differently depending on the context. Regeneration covers processes
487 from tissue repair to asexual reproduction. Basically, all these processes result in the same outcome, which is the
488 recreation of damaged or lost cells, tissues, organs and even entire body parts without the formation of scar tissue
489 and with complete functional integration [108]. Many organisms possess good regenerative capacities in early life
490 stages, especially as embryos or larvae, but lose this ability during metamorphosis, puberty and ageing [109].
491 However, some organisms maintain excellent regenerative capacities throughout their adult lives, being able to
492 regrow certain tissues, organs or even complete structures (limbs, lens, retina, spinal cord, brain, heart, and
493 neurosensory cells) [109, 110]. This lifelong ability to regenerate is widespread in the metazoan phyla, ranging
494 from invertebrates such as flatworms, cnidarians (*Hydra*), Arthropoda and Asteroidea (star fish) to vertebrates
495 including zebrafish, newts, clawed frog, axolotl and even mammals such as deer and spiny mice [110-113].

496 The regeneration process generally proceeds in three main phases: wound closure, cell proliferation/differentiation
497 and growth [111, 112]. After injury, wound closure is characterized by muscle contractions, filament re-
498 organization and the formation of a wound epithelium. In the second phase, new cells are necessary to re-establish
499 the lost tissue or structure. An orchestra of perfectly timed signalling factors maintains a correct balance between
500 cell proliferation, differentiation and apoptosis, guiding the acquired cells to their correct destination. All of these
501 processes are subjected to the redox balance, although the exact function of the redox signature during regeneration
502 is still being investigated. Many *in vitro* and *in vivo* studies already demonstrated the importance of ROS signalling
503 in the context of neuroregeneration. For example, ROS production has been associated with neuronal
504 differentiation in neuronal stem cells and with the induction of neurite outgrowth in hippocampal cell lines [114-
505 118]. Improved regenerative capacities of mechanosensory axons were discovered after an inactivating mutation
506 in an extracellular peroxidase gene (*i.e. pxn-2*) in *C. elegans*, [119]. Rieger and Sagasti showed that increased
507 hydrogen peroxide levels at the wound site are necessary for peripheral sensory axon regeneration following skin
508 injury in zebrafish larvae and that an inhibition of DUOX 1 resulted in impaired fin regeneration [120]. Lately
509 more and more publications indicate the importance of ROS signalling for successful regeneration of complete
510 body structures [121-123]. Both Love and Gauron [121, 122] published in Nature about the importance of a ROS
511 burst at the wound site for proper tail and fin regeneration of *Xenopus* tadpoles and zebrafish, respectively [121,
512 122].

513 It is hypothesized that ROS signalling modulates the regeneration process through the regulation of three crucial
514 cellular processes: proliferation, apoptosis and differentiation. Perfect orchestration of these processes results in
515 the formation of a blastema, a regenerative structure of newly differentiated cells that will give rise to the newly
516 formed structure [112]. ROS are necessary for blastema formation, since an inhibition of ROS production results
517 in a significant reduction in the blastema size as well as a diminished number of mitotic cells after the inhibition
518 of ROS production. Love and colleagues investigated the signalling pathways controlling cell proliferation, and
519 showed that increased ROS levels are necessary for the activation of the Wnt/ β -catenin signalling pathway, which
520 confers polarity and controls cell fate, as well as for one of its main downstream targets, *fgf20*, during posterior
521 regeneration [122, 124, 125]. During the developmental process, activation of the Wnt/ β -catenin pathway via ROS
522 signalling is modulated through NRX, a small redox-sensitive protein (see 1.4.1: ROS & patterning). It is likely

523 that ROS-induced activation of the Wnt/ β -catenin signalling pathway during regeneration also acts via NRX.
524 However, this has yet to be confirmed.

525 Apoptosis is a redox-controlled process and Gauron and colleagues were the first to link ROS production and the
526 induction of cell death in a regenerative context [121]. They showed that NOX inhibition reduces the number of
527 apoptotic cells and inhibits JNK activation. Exposure to inhibitors of ROS production, apoptosis, and JNK all
528 significantly reduce the number of proliferative cells and lead to impaired regeneration. These data suggest that
529 ROS signalling is necessary for the activation of the cell death process and JNK cascade, which work in parallel
530 to coordinate successful regeneration. MAPKs also influence cell differentiation. For example, during planarian
531 regeneration, JNK and ERK regulate the differentiation process. Inhibition of these enzymes blocks the cell cycle
532 and results in a reduced blastema formation [126-129]. Although MAPKs are shown to be redox-controlled in
533 various cellular and physiological processes, it still needs to be clarified whether ROS are the upstream triggers of
534 MAPK activation during regeneration.

535 Both ROS production and innervation seem to be crucial factors to achieve successful regeneration. However,
536 little is known about the crosstalk between both signalling processes [130, 131]. It has been shown that ROS
537 regulate neuroregeneration and differentiation [114-120]. Nevertheless, the question remains whether the general
538 impairment of the regeneration process is a direct result of ROS inhibition or if it is induced through the inhibition
539 of neuroregeneration and a lack of innervation. Removal or manipulation of nerve cords leads to incorrect
540 regeneration in various invertebrate and vertebrate organisms [132, 133]. For example, Endo demonstrates the
541 importance of the nervous system during newt ectopic limb regeneration and shows that denervation before
542 amputation/injury or in the early phase of blastema formation induces regression of the blastema and inhibits
543 successful regeneration [111]. To find the answers concerning the cross-talk between the redox balance and the
544 nervous system, more information must be gathered about the molecular mechanisms through which they both
545 regulate the regeneration process. Different regulation and activation of redox-associated pathways, including the
546 MAPK cascades, result in dissimilarities in neuroregenerative capacities between peripheral neurons, which have
547 the ability to regenerate after injury in most invertebrate and vertebrate organisms [134, 135]. Considering their
548 importance in the regulation of cell proliferation, differentiation, survival and migration, it is not surprising that in
549 various carcinogenic conditions, deregulation of the MAPK pathways is observed (discussed in the introduction
550 section) [31]. Studying redox-controlled pathways that regulate the regeneration process and carcinogenesis,
551 including the MAPK cascades, will provide crucial information to improve ROS-related therapies.

552 **3.1. Regeneration and cancer: following similar pathways to different destinations**

553
554 Cell proliferation, differentiation, death and migration are important processes during both regeneration and
555 cancer. While they are well coordinated during the regeneration of lost body parts, which is a normal physiological
556 process, the disease cancer is characterized by an uncontrolled execution of these processes and a failure to heal.
557 The initial signals, such as the capacity to sense damage and activate cell proliferation, are most likely unimpaired,
558 while the later events of regeneration, including tissue formation, remodelling, and the production of termination
559 signals, are lost during cancer [61, 108]. The regenerative environment is therefore suggested to suppress
560 carcinogen-induced malignant transformation [61, 108]. Animals with high regenerative capacities, such as

561 urodeles and planarians, functionally repair induced damage and regenerate over and over without malignant
562 transformations [108, 136, 137]. Newts, for example, successfully regenerate limbs and lenses many times over a
563 relatively short period, and even when chemical carcinogens are injected locally within the newt eye during lens
564 regeneration, the dorsal iris (capable of lens regeneration) does not form abnormal cells and is resistant to tumour
565 formation. On the contrary, in the ventral iris (non-regenerating) an exposure to carcinogenic compounds results
566 in abnormal cells that give rise to aggressive tumours [61, 108]. This was again confirmed in the planarian
567 *Dendrocoelum lacteum*, in which carcinogenic exposure of regenerating tissues induced mild differentiated
568 hyperplasia, whereas infiltrating tumours emerged in non-regenerating tissues. The regenerating tissue was even
569 able to reverse malignancies into differentiated structures and regain morphostasis [61, 108, 136, 137]. Although
570 the absolute numbers of cell division in newt or planarian regeneration do not reach those seen during normal
571 cellular turnover of, for example, mammalian skin over a lifetime, potent tumour suppressive functions must be
572 present during the regeneration process of these organisms. However, as the entire organism remains susceptible
573 to cancer, though often with a lower incidence, the ability to control tissue morphogenesis and impair unrestrained
574 proliferation and tumour formation seems to be a property of actively regenerating tissues and their
575 microenvironment [61]. In the highly regenerative mammalian liver, 95% of carcinogen-induced tumours also
576 remodel into normal tissue [137]. Thus, fundamental differences between regeneration and cancer lie in the
577 coordination of responses and in gene expression patterns rather than in structural or species-specific capacities
578 [108, 138]. Indeed, the underlying molecular and cellular mechanisms of these processes display a lot of
579 similarities. Between renal regeneration and repair and renal cell carcinoma for example, 77% of the differentially
580 changed genes were found to be concordant [138]. Therefore, a comparison of regenerative and carcinogenic
581 processes could reveal redox-controlled pathways that regulate, co-regulate or initiate cell proliferation,
582 differentiation, death and migration. In the next paragraphs, common redox-controlled molecular, cellular and
583 mechanistic contributors of both events will be discussed. A special emphasis will be put on stem cells. These cells
584 are important for regeneration and can transform into cancer stem cells which are essential in the building and
585 supporting of tumour microenvironments, in providing metastatic niches and in maintaining cancer hallmarks [99,
586 139, 140].

587

588 3.1.1. Genes

589 Perhaps one of the best characterized groups of genes which link cancer with regeneration is the family of tumour
590 suppressor genes. These genes regulate proliferation-dependent and possibly also proliferation-independent
591 aspects of regeneration [61]. Tumour suppression may therefore be an ancestral mechanism which surveys changes
592 in the environment, such as loss of tissues, to orchestrate the appropriate proliferation and differentiation responses
593 that are needed (wound repair or regeneration) while suppressing tumourigenesis [54, 61]. Abundance of these
594 regulatory molecules permits cell cycle re-entry from a quiescent state in early life stages, but works restrictive as
595 the organism ages, thereby limiting its regenerative abilities [109]. The function of tumour suppressors varies with
596 developmental stage and age within a species, and variable components are present in different species. Important
597 tumour suppressors involved in carcinogenesis and regeneration programs are Rb, p53, PTEN and Hippo. Rb and
598 p53 prevent inappropriate proliferation and eliminate or enable repair of stressed or genome-damaged cells, which
599 is important for ensuring a healthy and tumour-free regenerate [61]. A crucial role of Rb in the transition from a
600 proliferative to a post-mitotic differentiated state has been demonstrated in developmental studies in mice, where

601 the absence of Rb resulted in a failure of organized haematopoiesis, neurogenesis and myogenesis [141, 142].
602 Regulation of p53 is critical for proper limb regeneration in salamanders [143], imaginal disc regenerative potential
603 in *Drosophila* [144] and in axonal and liver regeneration in mice [145, 146]. The tumour suppressors PTEN and
604 Hippo are important in restraining and organizing tissue growth [61]. PTEN is an important negative regulator of
605 the PI3K signalling pathway, which constrains cell size, cell number and survival and is among the most frequently
606 inactivated tumour suppressor genes in human cancer [147, 148]. PTEN-dependent pathways are involved in axon
607 regeneration after injury and control β -cell regeneration in aged mice [149, 150]. In planarians, PTEN deficiency
608 results in the impairment of stem cell differentiation and inhibits regeneration [151]. The Hippo signalling pathway
609 is a more-recently-identified growth control pathway, which negatively regulates yorkie or its mammalian
610 homolog YAP (yes-associated protein 1) which is overexpressed in various cancers [61, 152, 153]. During
611 intestinal regeneration, the Hippo pathway controls YAP to allow compensatory proliferation while preventing
612 malignant transformation [153]. A knockdown of the Hippo pathway in the flatworm *Macrostomum lignano*
613 results in aberrant regeneration [154].

614 Other genes important in both cancer and regeneration are EGFR encoding genes. EGFRs regulate biological
615 processes such as cell proliferation and differentiation and are important in carcinogenesis to support tumour
616 growth, differentiation, survival and angiogenesis [155-158]. This signalling pathway is also required in
617 regeneration, for example in adult midgut epithelial regeneration in *Drosophila* and liver regeneration in mice
618 [159, 160]. Silencing of EGFRs during regeneration of the planarian *Schmidtea mediterranea* resulted in smaller
619 blastemas, abnormal differentiation of cephalic ganglia, decreased regeneration of eye pigment cells and abnormal
620 pharynges and mouth openings [155]. Examples of other interesting genes and pathways involved in both
621 processes are β -catenin [161-163], mTOR [164], the transforming growth factor β (TGF β)/bone morphogenetic
622 protein (BMP) and JAK/STAT [155].

623 3.1.2 Mechanisms

624 Cancer and regeneration are not only linked at the molecular level, they also share common mechanisms and
625 pathways. Important mechanisms during cancer and regeneration are the epithelial-mesenchymal transition (EMT)
626 and the reverse mesenchymal-epithelial transition (MET). These processes are active in various types of cancer
627 and during wound repair and tissue regeneration of adult tissues [66, 165-167]. A critical molecular event during
628 EMT is the loss of E-cadherin, a key component of adherens junctions. This loss releases β -catenin into the cytosol
629 and elicits activation of the canonical Wnt signalling pathway, again important in both regeneration and cancer
630 [163, 165].

631 Secondly, disturbances in gap junction-permeable signals and gap junction-mediated cell communication play a
632 role in carcinogenesis and are linked with developmental and regeneration disorders during embryonic
633 morphogenesis [137, 168-171]. In mice, the expression of specific connexins is upregulated during skeletal muscle
634 regeneration [104]. Regenerating planarians exposed to gap-junction blockers regenerate two heads [172, 173].

635 It has long been known that the immune system and inflammation are linked with cancer, but recently more and
636 more research also illustrates the importance of the immune system in the regeneration process [174-178]. Cells
637 of the immune system remove damaged cells and cell debris, produce signalling and growth factors, regulate
638 angiogenesis and modulate the extracellular matrix environment in the first stages after injury. Herein, ROS play

639 a crucial role, acting both as antimicrobials as well as signalling molecules [5]. Loss of immune cells, such as
640 macrophages, results in impaired regeneration [176, 177]. In addition, there is a reverse correlation between
641 immunity and regeneration abilities, since a decreased regenerative capacity was linked with the maturation of the
642 immune system in various vertebrate organisms [178, 179].

643 Regeneration and carcinogenesis share many similarities as to redox-controlled processes and mechanisms, yet
644 have different outcomes (Fig. 3). Now the question arises: can ROS be the controlling “agents” we are looking for
645 to overcome or even avoid cancer formation? A redox burst initiates and regulates both wound healing and
646 regeneration. After the regeneration process is completed, ROS levels decrease until normal physiological levels
647 are reacquired. Altered ROS levels are also observed during carcinogenesis, but the redox balance remains
648 disturbed throughout the entire duration of tumour formation [180]. Unfortunately, it is not a simple prolonged
649 redox status that leads to cancer. Although many cancer treatments based on alterations in the redox balance
650 produce successful results, they often lack specificity and have damaging side effects on healthy tissues. Knowing
651 the similarities and differences in redox control between regeneration and carcinogenesis will open doors to the
652 fine-tuning of pre-existing therapies and the development of novel diagnostic and therapeutic targets not only to
653 overcome, but also to prevent tumour development (Fig. 3).

654 **4. Redox-related anticarcinogenic targets in regenerative mechanisms**

655

656 **4.1. Direct redox-related anticarcinogenic targets**

657 The different redox signature of cancer cells and non-cancer cells is an opportunity to selectively target cancer
658 cells with redox-based cancer therapies (Fig. 3). Both ROS-elevating and -eliminating strategies have been
659 developed against cancer cells, since modest ROS levels enable tumour growth and survival, while excess levels
660 inhibit tumour cell proliferation and induce apoptosis, autophagy and necrosis. These strategies are reviewed by
661 Gupta *et al.* [85], Glasauer *et al.* [181] and Wondrak *et al.* [182].

662 Pro-oxidant based therapies elevate ROS levels by using ionizing radiation, ROS-generating chemotherapeutic
663 agents or agents that interfere with the antioxidant system. They are based on the fact that cancer cells already
664 possess elevated ROS levels and that a further increase in ROS will induce a redox shift resulting in cancer cell
665 death, the inhibition of cancer cell proliferation or motility. Since normal cells generally have higher capacities to
666 cope with additional ROS-producing insults than cancer cells, it is possible to preferentially accumulate ROS in
667 cancer cells and kill them selectively [1, 33, 85, 87, 182-184]. ROS-depleting strategies, on the other hand,
668 decrease ROS levels by using antioxidants, enhancers of ROS scavenging enzyme activities or NOX inhibitors.
669 They are based on the fact that ROS signalling is important in cancer cell proliferation and that a disturbance of
670 this signalling inhibits tumour growth. Most strategies, however, work through ROS generation and the consequent
671 induction of cell death [1, 33, 85, 183, 184]. Table 1 represents a few of the most described therapeutic procedures
672 of ROS elevating and eliminating strategies, which will be briefly illustrated below.

673 **4.1.1. ROS elevating strategies for cancer therapy**

674

675 One of the main strategies of ROS-elevating therapies is pushing the cancer cells into cell death. A subdivision is
676 made between agents that elevate ROS directly and agents that interfere with the antioxidant system (Table 1).
677 Procarbazine, for example, was one of the first drugs developed based on its hydrogen peroxide production and is
678 approved for the treatment of Hodgkin's lymphoma, non-Hodgkin's lymphoma, malignant melanoma and for
679 primary brain tumours (Table 1) [85, 185, 186]. Other ROS-generating compounds not mentioned in table 1, but
680 also used in cancer therapy or promising as novel therapeutic agents are, among others, imexon [187], elesclomol
681 273 [182, 188], bufalin [189], the natural alkaloid piperlongumine [190-194], hirsutanol A [183, 195, 196], the
682 fatty acid derivative trans-10, cis-12 conjugated linoleic acids [183, 197, 198], andrographolide [199-201], emodin
683 [202-204] c-phycoyanin [33, 205], bortezomib [206] and the vitamin E analogue tocopheryl succinate [33]. They
684 mainly induce apoptotic cancer cell death, but also affect cancer cell proliferation, angiogenesis and metastasis

685
686 Aside from ROS-generating compounds, radiotherapy also uses ROS to eliminate cancer cells as demonstrated in
687 a number of preclinical and clinical studies [85]. Furthermore, there are also many promising chemotherapeutic
688 agents that indirectly increase ROS levels as well, which can lead to apoptosis, inhibition of angiogenesis and
689 metastasis, or reduced proliferation in various cancers (Table 1).

690
691 Besides being used as monotherapy, ROS-generating agents are currently combined with chemo- and radiotherapy
692 to increase the vulnerability of cancer cells to these treatments [85, 146]. The nutraceutical curcumin possesses
693 both antioxidant and pro-oxidant activities, depending on the concentration and cancer type, and induces ROS-
694 dependent inhibition of proliferation as well as death of chemoresistant cells by sensitizing them to chemotherapy
695 [85, 182, 207]. A combination of curcumin with tamoxifen correlates with an increase in ROS generation and
696 results in synergistically induced cell death, both apoptosis and autophagy, in chemoresistant melanoma cells,
697 without affecting noncancerous cells [208]. Other potential therapies that are used to sensitize chemo- or
698 radioresistant cancer cells via ROS generation include triptolide [209], 2-methoxyestradiol [210], emodin [211],
699 D-allose [212], withaferin A [213], and As₂O₃ [214].

700

701 4.1.2. ROS eliminating strategies for cancer therapy

702 Agents with the potential to inhibit ROS generation are currently under development or used as cancer therapy,
703 although less frequently than ROS-elevating agents. These agents induce cancer cell death, suppress proliferation,
704 angiogenesis or metastasis. As for the ROS-elevating strategies, ROS can be eliminated directly or by acting upon
705 the antioxidant system (Table 2). The effectiveness of ROS-decreasing therapies in cancer treatment is
706 demonstrated in experimental studies in which overexpression of MnSOD decreases the proliferation of androgen-
707 independent human prostate cancer cells. Overexpression and/or delivery of other compounds of the antioxidant
708 system, such as glutathione peroxidase, catalase or SOD enzymes, has likewise been proven to be effective in
709 animal models (Table 2) [215-218]. ROS scavengers, such as the precursor of cysteine glutathione, N-acetyl-
710 cysteine (NAC), have also been proven to be effective as NAC is correlated with a decreased proliferation of
711 glioma cells (Table 2) [219]. A direct reduction of ROS production, for example via the inhibition of NOX
712 enzymes, similarly increases apoptosis, as shown in prostate and pancreatic cancer cells, and inhibits angiogenesis
713 and cancer cell invasion (Table 2) [88, 220-222].

714 Given the importance of a balanced redox state in the regulation of proliferation, cell death and migration,
715 antioxidant therapies are also incorporated into conventional chemotherapeutic or radiation treatment protocols to
716 scavenge therapy-induced ROS and, in this way, protect healthy cells and tissues [85, 223, 224]. Amifostine is an
717 example of such a free-radical scavenger drug that protects normal but not malignant cells against therapy-induced
718 ROS [225].

719 In conclusion, despite all the positive effects of redox-based therapy on tumours, there are still some major
720 concerns regarding their use [87, 181, 182]. First of all, as ROS regulate numerous signalling pathways, these
721 therapies can therefore be toxic for the surrounding tissues where they generate ROS-induced off-target effects on
722 the proliferation, migration and survival of healthy cells. As such, combinatorial antioxidant therapies that serve
723 as cytoprotective adjuvants are used to prevent cancer therapy-associated organ toxicity [182, 226]. Secondly,
724 ROS-induced biochemical and molecular changes may contribute to the emergence of drug resistant machineries
725 of cancer cells during disease progression. For example, increased resistance of multi-drug resistant leukaemia
726 cells to cytotoxic effects of hydrogen peroxide was found to be mainly caused by elevated catalase levels. The
727 resistance of bladder cancer cells to arsenic trioxide was associated with elevated SOD activity and reduced GSH
728 content [85]. Thirdly, improvident interference with the redox balance can have undesirable outcomes. For
729 example, while antioxidant supplementation reduces ROS and DNA damage, it prevents p53 activation and
730 increases tumour cell proliferation and tumour growth in mice [227]. Finally, the heterogeneous cell population of
731 tumours, consisting of cancer cells and cancer stem cells with different redox states, impedes a general redox-
732 altering approach. To tackle these problems, we should obtain further insights into redox-related signalling
733 pathways and regulatory networks that control cell proliferation, death and migration. A combination of direct
734 redox-altering therapies with therapies that target indirect redox-related processes can enhance drug efficiency and
735 specificity. In this regard, interesting targets can be searched within the regeneration process, in which cell
736 proliferation, death and migration are efficiently controlled and, presumably, redox-regulated.
737

738 **4.2. Indirect redox-related anticarcinogenic targets**

739 The redox state not only affects cell proliferation, death, and migration directly, it also alters these processes
740 indirectly through the regulation of molecular or systemic pathways. In the following sections, we will focus on
741 the indirect redox-related targets that are promising for anticarcinogenic therapies.

742 *4.2.1. Cellular redox-related targets*

743 *Tumour suppressor genes as therapeutic targets*

744 Probably one of the most studied tumour suppressor genes in cancer research is p53. This tumour suppressor and
745 nuclear transcription factor is important for the induction of cell cycle arrest, as well as for DNA repair, senescence,
746 apoptosis, autophagy and the metabolism [228-230]. In most tumours p53 is hijacked, either by mutations or
747 deletions that disrupt its DNA-binding activities and prevent the consequent transcriptional transactivation of
748 target genes, or by posttranslational modifications or cytoplasmic sequestrations that disturb p53 activity [228,
749 230, 231]. In addition, inactive p53 pathways in cancer cells are associated with a higher resistance to conventional

		Mechanism	Compound	Therapeutic use	References	
ROS ELEVATING STRATEGY	Direct agents	H ₂ O ₂ production	Procarbazine	(non)-Hodgkin's lymphoma, malignant melanoma, primary brain tumours	[85, 185, 186]	
		Production of superoxide anions via NOX activation	Parthenolide and its analogue dimethylaminoparthenolide	Induction of apoptosis and growth suppression in prostate cancer, primary leukaemia, osteosarcoma, melanoma and triple-negative breast cancer	[85, 182, 232, 233]	
		Elevation of superoxide levels by impairing mitochondrial electron transport chain	Arsenic trioxide	Used for treatment of leukaemia	[1, 85, 182, 184, 234, 235]	
	Agents interfering with the antioxidant system	Thioredoxin inhibitor	Motexafin gadolinium	Induces apoptosis; in trial for chronic lymphocytic leukaemia (phase II) and non-small cell lung cancer with brain metastasis (phase III)	[85, 182, 184, 236]	
		GSH inhibition via γ -glutamylcysteine synthetase inhibition	Buthionine sulfoximine (BSO)	Combined with arsenic trioxide for treatment of advanced solid tumours	[85, 237-241]	
		GSH inhibition via inhibition of GPX and GSH conjugations	β -phenylethylisothiocyanate (PEITC)	Selectively induces cancer cell death while being less toxic to normal cells	[1, 33, 85, 182, 239-244]	
		SOD1 inhibition	ATN-224	Inhibits cancer cell proliferation and metastasis. Phase II trial for recurrent prostate cancer, solid tumours, multiple myeloma and resistant malignancies <i>in vitro</i>	[1, 33, 85, 182, 239, 242-246]	
		SOD1 inhibition	2-methoxyestradiol	Phase I trial for metastatic breast cancer, phase II for prostate cancer	[85, 182, 245, 246]	
		Chemotherapeutic agents that also increase ROS levels	Garlic diallyldisulfide & diallyltrisulfide		Cell cycle arrest and apoptosis in colon and lung cancer	[182, 247, 248]
			Dasatinib + oxaliplatin combination		Reduce proliferation and angiogenesis in colon cancer cells	[249]
Theaflavins and berberine			Inhibition of the metastatic potential	[250, 251]		

750 **Table 1: Overview of direct redox-related anticarcinogenic strategies elevating ROS levels**

		Mechanism	Compound	Therapeutic use	References		
ROS ELIMINATING STRATEGY	Direct	ROS suppressors	Inhibitors of ROS-generating NOX enzymes	Increase in apoptosis of pancreatic and prostate cancer cells and decrease in angiogenesis and invasion	[88, 220-222]		
			XQ2	Growth inhibition of non-small-cell lung carcinoma	[252]		
	Via the antioxidant system	Overexpression of MnSOD	Experimental studies: decrease in proliferation of androgen-independent human prostate cancer cells			[215, 216]	
		Overexpression of glutathione peroxidase					
		Transgenic overexpression/delivery of catalase	Reduced tumour aggressiveness in mouse metastatic breast cancer model/inhibition of metastasis to liver, lung and peritoneal organs			[217, 253, 254]	
		Delivery of PEG-conjugated SOD enzymes	Inhibits peroxidation and metastatic tumour growth in mice			[218]	
		Antioxidants (scavengers)	Fullerene	Inhibition of growth and metastasis in a mouse breast cancer model			[98]
			N-acetyl-cysteine	Decreased proliferation of glioma cells			[219]
	Amifostine		Protects normal but not malignant cells against therapy-induced ROS			[225]	

751 **Table 2: Overview of direct redox-related anticarcinogenic strategies eliminating ROS**

752
753 chemo- and radiotherapy [228, 231, 255]. Therefore, reactivating suppressed p53 or rescuing mutant p53 in
754 tumours may trigger lethality or permanent growth arrest in p53-deficient cancer cells, which is a promising
755 strategy for successful anticancer treatment. Since p53 also protects normal tissues against off-target effects of
756 cancer therapies, p53 restoring therapies also bypass deleterious side effects resulting from most of the current
757 cancer treatments [228, 231, 255, 256]. For many years now, the modulation of p53 for cancer therapy is a very
758 active area of research [255]. For example, wild-type p53 functions in humans are restored via the use of virus-
759 mediated p53 gene replacement [231, 255, 256]. Adenovirus-based p53 gene therapies have been approved by the
760 Chinese government for the treatment of head and neck carcinomas, in which p53 mutations are frequent and
761 increase with progression of the disease [231, 256]. Another way to restore p53 function is by pharmacologically
762 activating wild-type p53, for example by blocking murine/human double minute 2 (MDM2) or its human
763 counterpart HDM2, negative regulators of p53 stability. HDM2 is overproduced in many human tumours as a
764 mechanism to restrict p53 function, leading to inefficient growth arrest and apoptosis. The small molecule
765 compound RITA (reactivation of p53 and induction of tumour cell apoptosis) and the nutlin RG7112 are examples

766 of compounds that activate p53 via these regulators and induce apoptosis and cell cycle arrest specifically in cancer
767 cells without initiating DNA damage in normal cells [229, 231, 255, 256]. A restoration of the wild-type
768 conformation and DNA binding capacities of mutant p53 is achieved by systemic administration of PRIMA-1 (p53
769 reactivation and induction of massive apoptosis) and its structural analogue PRIMA-1Met (APR-246), which
770 induce apoptosis and inhibit tumour growth *in vivo* with a high degree of target specificity [228, 231]. Both
771 compounds have been tested in primary leukemic cells from acute myeloid leukemia and chronic lymphocytic
772 leukemia patients and APR-246 is being tested in a phase I clinical trial of patients with haematological
773 malignancies or prostate cancer [256]. Other treatments that target p53 are reviewed by Chen *et al.* [231].

774
775 Limitations of p53-based cancer treatments are reviewed by Miryazans *et al.* [257] and include the indirect
776 targeting of downstream effectors of the p53 pathway, which could interfere with the therapy efficiency or even
777 worsen the outcome. For example, p21, a downstream target in the p53 pathway, induces cancer cell senescence,
778 which is thought to trigger the secretion of growth-stimulating and metastasis-inducing factors. By targeting p53,
779 p21-induced senescence could promote the growth of neighbouring cancer cells. For the same reason, tumour cell
780 heterogeneity, which results into a wide variety of responses within a tumour, could limit p53-based cancer
781 treatments. A last critical challenge of p53-targeted therapies is the elimination of cancer stem cells that drive
782 abnormal tumour growth and exhibit a high resistance to cytotoxic effects of therapeutic agents. Especially at
783 advanced stages, the high-genetic plasticity of human tumours increases the chance for acquired resistance to most
784 single-agent therapies. Therefore, combinatory approaches in cancer therapy are often necessary and are
785 extensively sought after. Since p53 is regulated in a redox-dependent manner and exerts its effects on cell
786 proliferation and cell death via regulation of the cellular redox state, combinatorial therapies that target the redox
787 balance could improve their efficiency. Redox-related anticarcinogenic targets and their effects on stem cells
788 should be studied within the controlled environment of regeneration [228, 256].

789 790 *Signalling pathways as a therapeutic target*

791 Many signalling pathways that regulate cell proliferation, cell death, differentiation and migration are controlled
792 by the cellular redox state. As such, the redox-regulated transcription factors HIF-1, NF- κ B, and Nrf2 are potential
793 therapeutic targets considering their roles in malignant tumour progression. A subunit of HIF, named HIF-1 α ,
794 supports cancer cell proliferation and chemoresistance and inhibitors of this subunit, such as PX-478, are currently
795 tested in clinical trials for anticancer activities [182]. NF- κ B is also activated in various types of cancer and
796 regulates proliferation, suppression of apoptosis and migration. NF- κ B induces dedifferentiation of non-stem cells
797 through modulation of the Wnt signalling pathway, resulting in the acquirement of tumour-initiating
798 capacities. Inhibitors of the NF- κ B pathway, including IKK blockers (BAY-11-7082, BAY-11-7085, MLN120B),
799 proteasome inhibitors (Bortezomib) and NF- κ B DNA-binding inhibitors (SN-50), are currently in clinical use as
800 cancer chemotherapeutics, whereas others are undergoing clinical development and trials [258-261]. Nrf2 controls
801 the antioxidant defence system and promotes protection of cells against ROS-induced damage, thereby decreasing
802 the incidence of tumour initiation. In this context, Nrf2-related factors are potential chemopreventive targets [262,
803 263]. On the other hand, Nrf2 protects cells against apoptosis and inhibition of this factor, for example through
804 ingestion of active stilbenes in fruits and vegetables, induces cell death in malignant cells [264].

805 ROS not only directly target transcription factors, they also influence their activation through interference with
806 various signalling pathways. Alterations in signalling pathways such as MAPK cascades caused by abnormal
807 activation of receptor tyrosine kinases or gain-of-function mutations are observed in different types of cancer [265,
808 266]. These redox-regulated proteins are good potential targets for cancer treatment and various small molecule
809 inhibitors have been developed and are currently being tested in clinical trials [265, 267]. Inhibition of Ras, the
810 upstream activator of the ERK MAPK pathway, did not provide promising results, probably due to the
811 nonspecificity of these inhibitors. However, inhibition of MEK 1/2 or RAF both show interesting results as potent
812 anticancer therapeutics and many manufacturers are working to produce inhibitors with high specificity and
813 selectivity for different MAPK isoforms [31]. The possible inhibitors of the Ras/ERK MAPK signalling pathway
814 which are currently investigated are reviewed in detail in Santarpia *et al.* [265]. Not only the ERK pathway, but
815 also the p38 MAPK and JNK pathways offer interesting targets since they regulate apoptosis, cell proliferation,
816 and the inflammatory response. Just as with the direct redox-related targets, combinatorial treatment with these
817 therapies might trigger cancer cell death or increase the sensitivity of tumour cells to other therapeutic agents [31].
818 A possible reverse strategy is the activation of p38 MAPK, since this might sensitize the malignant cells to
819 apoptosis. Caution is however necessary since this activation might enhance the inflammatory response and
820 possibly cancer progression.

821 EGFR signalling is another redox-controlled signalling cascade involved in cell cycle progression [268-271].
822 Anticancer therapies frequently target mutated EGFR kinase, and inhibit processes involved in tumour growth and
823 progression [269, 270]. A number of monoclonal antibodies directed against its extracellular ligand-binding
824 domain were developed. For example, cetuximab and panitumumab block ligand-induced EGFR tyrosine kinase
825 activation, leading to inhibition of cellular proliferation and induction of apoptosis [269, 270].

826

827 The applied strategy (which signalling factor/pathway should be activated or deactivated) depends on the cancer
828 type and stage. A combination of these indirect redox-related therapies with existing redox-altering therapeutics
829 can enhance their efficiency. The precise control of cell proliferation, death and migration via these signalling
830 pathways during the regeneration process provides an interesting context to study the impact of redox interference
831 through these cascades on these processes. This knowledge will guide us towards the correct use of these targets
832 or discovery of new targets as anticarcinogenic therapeutics.

833 *Telomerase*

834 In 80-95% of the cancers, telomerase is overexpressed. Telomerase protects cancer cells against DNA damage,
835 cell senescence and the consequent induction of apoptosis, and as such promotes tumour growth. This enzyme is
836 also activated in adult stem cells during planarian regeneration [272]. This makes telomerase an ideal candidate
837 target for anticancer therapeutics. Several inhibiting strategies are being tested, including small molecule
838 inhibitors, antisense oligonucleotides, immuno- and gene therapies targeting the hTERT or hTER, G-quadruplex
839 stabilizers, tankyrase, and HSP90 inhibitors [273-275]. Based on the review of Ruden (2013), the most promising
840 therapies/therapeutic compounds are the antisense oligonucleotide inhibitor GRN163L and immunotherapies that
841 use dendritic cells (GRVAC1), hTERT peptide (GV1001) or cryptic peptides (Vx-001). These agents are currently
842 being tested in phase I and II clinical trials in patients with various types of cancers. Promising results were
843 obtained concerning the reduction of tumour growth inhibition, overall survival and disease stabilization, but

844 combinatorial therapies appeared the most effective [273]. Concerning this therapeutic strategy, combining
845 telomerase inhibition with pro-oxidant treatments also might result in accelerated telomere shortening, cancer cell
846 senescence/death and tumour regression.

847 4.2.2. System redox-related targets

848

849 *Metabolic factors as therapeutic targets*

850 Cancer cells have an altered metabolism, (based on aerobic glycolysis) to sustain their demand for large quantities
851 of proteins, lipids and nucleotides as well as for energy. They require NADH and NADPH for the correct
852 functioning of their antioxidant systems. Targeting these reducing molecules, by targeting the metabolism,
853 provides an indirect pro-oxidant therapeutic intervention [182]. This can be achieved by interfering with the
854 glutamine metabolism, for example through inhibition of glutaminase 1 (GSL1), or with related pathways such as
855 aspartate transaminase (GOT1), impairing GSH synthesis and inducing cell death [181]. Another interesting
856 enzyme in the metabolic pathways of cancer cells is p53-inducible glycolysis and apoptosis regulator (TIGAR),
857 the downstream target of p53. TIGAR regulates the redox balance in tumour cells by promoting NADPH
858 production, generating reduced GSH to support cancer cell survival and tumour growth. A decrease in the number
859 of tumours and a promotion of overall survival was shown in a TIGAR-deficient intestinal adenoma mouse model
860 [86, 276].

861 The metabolism can also be targeted indirectly through alterations in the activity of redox-regulated upstream
862 regulators of the metabolic pathways such as HIF, PI3K/Akt and AMP-activated protein kinase (AMPK).
863 Metformin, an AMPK activator, has anticarcinogenic effects in both mouse models of breast cancers and in breast
864 cancer patients. PI3K inhibitors reversed some of the metabolic phenotypes of cancer cells, resulting in tumour
865 regression. Insulin-like growth factor receptors (IGFRs) regulate various signalling cascades, including the
866 PI3K/Akt pathway, which makes them potential new targets for cancer therapy. However, it must be noted that
867 these signalling pathways not only control the metabolism, they also act as upstream regulators of non-metabolic
868 pathways. Studying the redox-regulated metabolic control and the precise roles of the upstream signalling factors
869 during regeneration will provide a complete picture of their regulation of proliferation and cell death to optimize
870 their function as specific anticarcinogenic targets.

871 *Inflammatory factors and the nervous system as therapeutic targets*

872 Although the immune and nervous system do not directly influence proliferation, differentiation or cell death, both
873 systems are redox-regulated and have important roles during the carcinogenic process, therefore providing
874 important targets for the development of anticancer drugs, which are discussed below.

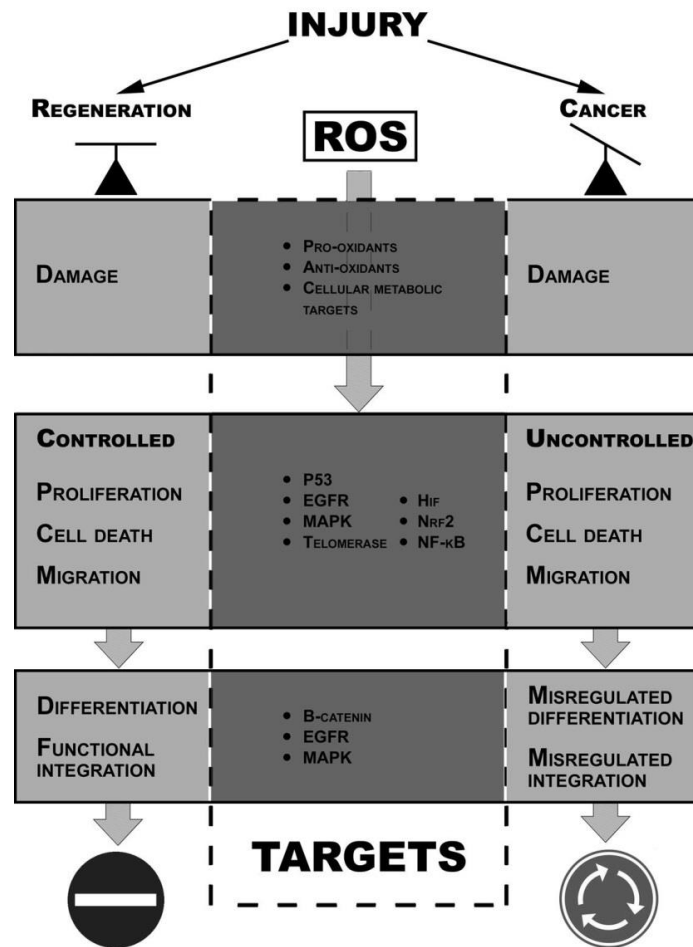
875 Leukocyte recruitment and activation of both the innate and the adaptive immune system are directly related to
876 tumour onset. Although not all the details are fully understood, various studies on long-term therapy with anti-
877 inflammatory drugs demonstrate their usefulness in inhibiting tumour progression and the development of
878 malignancies. Analyses performed by Rothwell and colleagues revealed that daily aspirin users had a significantly
879 lower risk of death from cancer (the 20-year risk of cancer death was reduced by 60% for gastrointestinal cancers
880 and by 30% on average for other solid tumour cancers, including oesophageal, pancreatic, stomach, lung, brain

881 and prostate cancers) in comparison to the group that did not receive a daily aspirin dose [277]. These analyses
882 confirmed the results of earlier studies, which indicated that daily use of aspirin and other non-steroidal anti-
883 inflammatory drugs (NSAIDs) reduced the risk for colorectal tumour formation and reoccurrence of these polyps.

884 The immune system can also be targeted via the activation of granulocytes. These cells are important suppressors
885 of tumour growth. Activated granulocytes produce ROS, thereby altering the redox environment of cancer cells,
886 and generate other cytotoxic factors, such as proteases and membrane-proliferating agents. Granulocytes are
887 recruited naturally into the tumour, but do not reach critical numbers to significantly affect the tumour via their
888 cytolytic activities. Their presence results in tumour progression and metastasis because of the cross-talk between
889 granulocytes and cancer cells. Augmenting the granulocyte activation and recruitment, which can be achieved by
890 treatment with granulocyte colony-stimulating factor, increases specific antibody dependent cytotoxicity and
891 decreases immunoresistance [259, 278].

892 Not only the immune system, but also the nervous system is involved in cancer development, as was indicated by
893 multiple studies. A process named perineural invasion (PNI) improves tumour growth and metastasis through
894 survival signals of the neurons and migration along the native nerve fibres [279, 280]. The adrenergic fibres from
895 the sympathetic nervous system were identified to be responsible for the promotion of tumour development and
896 survival via β 2- and β 3-adrenergic receptors, while cholinergic cells of the parasympathetic system promote
897 tumour cell invasion and migration [279]. The 5-year disease-free survival rate is four-fold larger for patients with
898 PNI-negative colorectal tumours in comparison to patients with PNI-positive tumours. The 5-year overall survival
899 rate is 72% for PNI-negative tumours versus 25% for PNI-positive tumours [281]. The perineural system can be
900 targeted by inhibition of the neurological functioning through intake of blockers that diminish tumour growth.
901 Recent epidemiological data suggest that β -blocker intake is associated with improved survival of prostate cancer
902 patients [279]. Targeting of the nerve growth factor (NGF) also inhibits tumour cell progression, survival and
903 migration in breast cancer [282]. Although further research is necessary, these data already demonstrate the
904 possibilities of drugs targeting both branches of the autonomic nervous system for cancer treatment.

905 The immune and nervous system are not only involved in carcinogenesis, they also come forward as important
906 factors during the regeneration process. Their role, however, is adverse. The evolutionary improvement of the
907 immune system in higher organisms, especially in vertebrates, resulted in the loss of regenerative capacities, while
908 presence of neuronal cells is crucial for regeneration to proceed [283]. Acquiring more insights concerning their
909 function in the regeneration process, and the role of the redox balance herein, in order to compare it with their
910 function during the carcinogenic process, can deliver new insights in potential mechanisms that can be targeted to
911 treat or even avoid tumour formation. In this way, not only new targets can be found, but also existing treatments
912 can be improved.



913

914 **Figure 3: Redox-related anticarcinogenic targets in regenerative mechanisms.** Regeneration and cancer share
 915 many similarities, but have very opposite outcomes. The redox signature modulates the responses of cells to injury
 916 in both processes. During regeneration, ROS signalling induces controlled cell proliferation, death and migration
 917 for successful formation of the missing tissue. When growth and functional integration into the existing tissues are
 918 completed, regeneration stops. However, during carcinogenesis the redox balance is disturbed and increased ROS
 919 levels will cause DNA damage and loss of control over these cellular processes, leading to unwanted tumour
 920 formation. Moreover, these tumour cells keep on dividing uncontrollably, ultimately resulting in death of the
 921 organism. Since the redox signature controls regeneration and carcinogenesis, both at initiation and during later
 922 stages, studying the role of the redox balance in controlling these mechanisms during regeneration, could provide
 923 interesting targets to control, overcome and even avoid cancer.

924 4.2.3. Cancer stem cells as a therapeutic target

925 Studying the process of regeneration is not only important in the abovementioned processes, it also delivers new
 926 insights into the underlying mechanisms of totipotency and pluripotency in stem cells, which again can be used in
 927 anticancer therapies, *e.g.* to target cancer stem cells. While conventional anticancer therapies predominantly attack
 928 the bulk tumour cell populations, cancer stem cells are often resistant to these therapies. As they seem to be the
 929 only fraction of tumour cells capable of initiating a new tumour, unmasking the factors that sustain cancer stem
 930 cell survival is necessary to develop more efficient therapies that reduce the risk of tumour relapse and metastasis

931 [284-286]. One of the upstream regulators of the various phases of the cancer process and of stem cell quiescence,
932 both during cancer and regeneration, is the redox balance. Understanding its exact role in the activation/regulation
933 of the specific stem cell processes offers opportunities towards new therapeutic strategies against cancer [284]. In
934 the following sections, examples of existing and promising therapies that target the redox balance of cancer stem
935 cells will be discussed.

936

937 Cancer stem cells often possess a higher resistance to radio- or chemotherapy as a result of higher defence
938 potentials against ROS. Adhesion molecules and stem cell markers, such as CD44 and its alternative mRNA
939 splicing variant CD44v, which stimulate the intracellular GSH levels, are examples of protection mechanisms of
940 cancer stem cells against high environmental ROS levels (as explained in section 2.3.2) [287]. An upregulation of
941 CD44 in cancer stem cells is believed to be responsible for the incurability of glioblastoma multiforme, the most
942 aggressive brain tumour. Therapies that target CD44, through RNAi or via administration of a monoclonal
943 antibody, are able to disturb the ROS defence of cancer stem cells and are potential therapeutic targets to kill
944 cancer stem cells in various tumours [1, 286, 287].

945 Another defence mechanism of cancer stem cells is the expression of multidrug resistance ABC transporters that
946 pump redox-altering drugs out of the cell. The chemoresistance mediator ABCB5 positively correlates with
947 neoplastic progression in human melanoma patients and is preferentially expressed by melanomas with high *in*
948 *vivo* tumorigenic capacity and of metastatic tumour origin. Administration of an anti-ABCB5 antibody
949 substantially inhibits tumour formation and growth in melanoma mice and overcomes resistance to the H₂O₂-
950 generating, chemotherapeutic agent doxorubicin [286].

951 Cancer stem cells also protect themselves against redox-altering chemotherapies and targeted therapies by
952 maintaining a quiescent state. The induction of differentiation in quiescent cancer stem cells is therefore a
953 promising approach to eliminate cancer stem cells. This can be achieved among others by administration of Notch
954 pathway inhibitors, which were shown to deplete medulloblastoma stem-like cells or by enforced expression of
955 miRNAs which induces differentiation of breast cancer stem cells and inhibits their tumour-forming ability in
956 mice. Doxorubicin and cyclophosphamide therapy in combination with an epigenetic therapy with the
957 demethylating agent hydralazine and the histone deacetylase inhibitor magnesium valproate also induces cancer
958 stem cell differentiation and improves clinical outcome of breast cancer patients [286]. Arsenic trioxide is thought
959 to eliminate cancer stem cells through degradation of PML, a tumour suppressor gene involved in the chromosomal
960 translocation of promyelocytic leukaemia as well as an important factor in maintaining the quiescent state of CML
961 cancer stem cells, and through increasing ROS levels [1].

962 Finally, the preferential activation of the DNA damage checkpoint response and increased DNA repair capacity
963 which can diminish therapy-induced ROS damage is thought to increase the radioresistance of cancer stem cells.
964 An inhibition of the Chk1 and Chk2 checkpoint kinases for example reversed the radioresistance of CD133+
965 glioma cancer stem cells [286].

966

967 Not only stem cells but also the tissue microenvironment, consisting of resident and infiltrating host cells and
968 components of the extracellular matrix, play an essential role in regeneration, cancer development and the

969 modulation of cell responses to cancer treatments [95, 287]. The niche of cancer stem cells controls their self-
970 renewal, proliferation, differentiation and apoptosis and protects them against genotoxic insults. In addition, it can
971 generate cancer stem cells through induction of stem-like features in more differentiated tumour cells by
972 reactivating the Wnt pathway. The niche of cancer stem cells is also involved in metastasis by inducing EMT and
973 by formation of a premetastatic niche for secondary tumours. The niche and its mediators are therefore also used
974 as targets for anticancer therapy [95, 287]. An example are the anti-MET antibodies that inhibit hepatocyte growth
975 factor, produced by the microenvironment, to bind to the tyrosine kinase receptor MET, and in this way inhibit
976 colon cancer growth [285].

977 **Conclusion**

978 ROS signalling is involved in multiple cellular processes including cell proliferation, cell death, cell differentiation
979 and cell migration through which they regulate correct physiological responses and tissue regeneration. A
980 controlled redox balance is also crucial to maintain self-renewal capacities and to regulate stem cell fate.
981 Disturbances in this balance, induced by either endogenous or external factors, initiate many pathological
982 conditions including neurodegeneration, immunity disorders and cancer. In tumours, ROS are not only important
983 for the induction of DNA damage in proto-oncogenes, oncogenes or tumour suppressor genes, disturbances in the
984 redox signature also support cancer cell survival through altering signalling pathways, affecting the
985 microenvironment and promoting migration and metastasis. Therefore, cancer cells and cancer stem cells regulate
986 ROS levels dynamically to enable this oxidative damage and malignant transformation while avoiding cell death.
987 Pro-oxidative mechanisms include upregulation of ROS-producing enzymes via tumour suppressor genes and
988 oncogenes, and stimulation of the metabolism. The activation of redox-sensitive transcription factors that
989 upregulate antioxidant ROS scavenging enzymes and promote metastasis are strategies of cancer cells to prevent
990 ROS levels to reach a cell death threshold. Many redox-based anticarcinogenic therapies that target the differential
991 redox state of cancer cells in comparison with non-cancer cells are currently being developed and applied.
992 Although they give positive outcomes concerning tumour regression and prevention of metastasis, these therapies
993 have disadvantages such as a lack of specificity and induction of drug resistance. Especially cancer stem cells,
994 which possess enhanced protection mechanisms against ROS-induced stress, are less vulnerable to redox-based
995 anticarcinogenic therapies, thereby increasing the chance of tumour relapse. To tackle these shortcomings of direct
996 redox-related therapies, better insights concerning the involvement of the redox balance as a regulator in cancer-
997 related processes should be obtained. In this regard, the regeneration process, which is a normal physiological
998 process in diverse animals and/or life stages, offers a unique context to study the importance of ROS signalling in
999 controlling cell proliferation, cell death and cell migration. Various genes and mechanisms that are essential in the
1000 establishment of successful regeneration have been shown to be disturbed in carcinogenesis, many of which are
1001 redox-controlled. These genes and mechanisms offer interesting indirect redox-related targets to fine-tune existing
1002 therapies. For example, therapeutics targeting tumour suppressor genes like p53 or MAPK cascades are promising
1003 for the treatment of cancer. In addition, new insights in behaviour and adaptive responses of stem cells to changing
1004 environments as well as during unsuccessful regeneration could reveal potential therapeutic targets against cancer
1005 stem cells.
1006

1007 Redox-related direct and indirect mechanisms are not only interesting in cancer treatment, but also in cancer
1008 prevention. Epidemiological studies that linked high consumptions of antioxidant-rich fruits and vegetables with
1009 a better health, have led to the assumption that dietary intake of antioxidants, such as curcumin, reduces ROS and
1010 ROS-associated diseases. Although people with certain genetic polymorphisms in antioxidant genes might benefit
1011 from these supplements, some caution must be exercised. An improvident use of antioxidants has been associated
1012 with a decrease in general health and even an elevated risk of mortality. The potential impeding effects of
1013 antioxidants with treatment success of radio- and chemotherapy, both depending on ROS to induce cytotoxicity in
1014 tumours, has also been a subject of discussion in the medical community.

1015
1016 In summary, redox-related therapies are interesting and active areas of on-going cancer research. However, more
1017 resources should be invested in studies that explore the basic defaults that occur in the initiation of cancer
1018 transformation. Since high regenerative capacities are associated with tumour repressing abilities, unravelling
1019 regeneration processes that suppress malignant transformation provide opportunities to overcome, treat and even
1020 avoid cancer.

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1041 **References**

- 1042 [1] Kobayashi CI, Suda T. Regulation of reactive oxygen species in stem cells and cancer stem cells. *J*
1043 *Cell Physiol* 2012; 227(2): 421-30.
- 1044 [2] Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. *Nature* 2000; 408(6809):
1045 239-47.
- 1046 [3] Dröge W. Free radicals in the physiological control of cell function. *Physiol Rev* 2002; 82(1): 47-95.
- 1047 [4] Buonocore G, Perrone S, Tataranno ML. Oxygen toxicity: chemistry and biology of reactive oxygen
1048 species. *Semin Fetal Neonatal Med* 2010; 15(4): 186-90.
- 1049 [5] Kanta J. The role of hydrogen peroxide and other reactive oxygen species in wound healing. *Acta*
1050 *Medica (Hradec Kralove)* 2011; 54(3): 97-101.
- 1051 [6] Brieger K, Schiavone S, Miller FJ, Jr., Krause KH. Reactive oxygen species: from health to disease.
1052 *Swiss Med Wkly* 2012; 142: w13659.
- 1053 [7] Covarrubias L, Hernández-García D, Schnabel D, Salas-Vidal E, Castro-Obregón S. Function of
1054 reactive oxygen species during animal development: passive or active? *Dev Biol* 2008; 320(1): 1-11.
- 1055 [8] Jiang F, Zhang Y, Dusting GJ. NADPH oxidase-mediated redox signaling: roles in cellular stress
1056 response, stress tolerance, and tissue repair. *Pharmacol Rev* 2011; 63(1): 218-42.
- 1057 [9] Fleury C, Mignotte B, Vayssiere JL. Mitochondrial reactive oxygen species in cell death signaling.
1058 *Biochimie* 2002; 84(2-3): 131-41.
- 1059 [10] Murphy MP. How mitochondria produce reactive oxygen species. *Biochem J* 2009; 417(1): 1-13.
- 1060 [11] Bienert GP, Moller AL, Kristiansen KA, *et al.* Specific aquaporins facilitate the diffusion of hydrogen
1061 peroxide across membranes. *J Biol Chem* 2007; 282(2): 1183-92.
- 1062 [12] Balaban RS, Nemoto S, Finkel T. Mitochondria, oxidants, and aging. *Cell* 2005; 120(4): 483-95.
- 1063 [13] Gupta RK, Patel AK, Shah N, *et al.* Oxidative Stress and Antioxidants in Disease and Cancer: A. *Asian*
1064 *Pac J Cancer Prev* 2014; 15(11): 4405-9.
- 1065 [14] Willcox JK, Ash SL, Catignani GL. Antioxidants and prevention of chronic disease. *Crit Rev Food Sci*
1066 *Nutr* 2004; 44(4): 275-95.
- 1067 [15] Halliwell B, Gutteridge J. Cellular responses to oxidative stress: adaptation, damage, repair,
1068 senescence and death. New York: Oxford University; 2007.
- 1069 [16] Copple IM, Goldring CE, Jenkins RE, *et al.* The hepatotoxic metabolite of acetaminophen directly
1070 activates the Keap1-Nrf2 cell defense system. *Hepatology* 2008; 48(4): 1292-301.
- 1071 [17] Kaspar JW, Niture SK, Jaiswal AK. Nrf2:Keap1 signaling in oxidative stress. *Free Radic Biol*
1072 *Med* 2009; 47(9): 1304-9.
- 1073 [18] Nguyen T, Nioi P, Pickett CB. The Nrf2-antioxidant response element signaling pathway and its
1074 activation by oxidative stress. *J Biol Chem* 2009; 284(20): 13291-5.
- 1075 [19] Tell G, Quadrioglio F, Tiribelli C, Kelley MR. The many functions of APE1/Ref-1: not only a DNA
1076 repair enzyme. *Antioxid Redox Signal* 2009; 11(3): 601-20.
- 1077 [20] Bhakat KK, Mantha AK, Mitra S. Transcriptional regulatory functions of mammalian AP-
1078 endonuclease (APE1/Ref-1), an essential multifunctional protein. *Antioxid Redox Signal* 2009; 11(3):
1079 621-38.
- 1080 [21] Liu B, Chen Y, St Clair DK. ROS and p53: a versatile partnership. *Free Radical Biology and Medicine*
1081 2008; 44(8): 1529-35.
- 1082 [22] Kops GJ, Dansen TB, Polderman PE, *et al.* Forkhead transcription factor FOXO3a protects quiescent
1083 cells from oxidative stress. *Nature* 2002; 419(6904): 316-21.
- 1084 [23] Alexander A, Kim J, Walker CL. ATM engages the TSC2/mTORC1 signaling node to regulate
1085 autophagy. *Autophagy* 2010; 6(5): 672-3.
- 1086 [24] Cosentino C, Grieco D, Costanzo V. ATM activates the pentose phosphate pathway promoting anti-
1087 oxidant defence and DNA repair. *EMBO J* 2011; 30(3): 546-55.
- 1088 [25] Ditch S, Paull TT. The ATM protein kinase and cellular redox signaling: beyond the DNA damage
1089 response. *Trends Biochem Sci* 2012; 37(1): 15-22.
- 1090 [26] Bubici C, Papa S, Pham CG, Zazzeroni F, Franzoso G. The NF-kappaB-mediated control of ROS and
1091 JNK signaling. *Histol Histopathol* 2006; 21(1): 69-80.

1092 [27] Janssen-Heininger YM, Mossman BT, Heintz NH, *et al.* Redox-based regulation of signal
1093 transduction: principles, pitfalls, and promises. *Free Radic Biol Med* 2008; 45(1): 1-17.

1094 [28] Winterbourn CC, Hampton MB. Thiol chemistry and specificity in redox signaling. *Free Radical Biol*
1095 *Med* 2008; 45(5): 549-61.

1096 [29] Zhang W, Liu HT. MAPK signal pathways in the regulation of cell proliferation in mammalian cells.
1097 *Cell Res* 2002; 12(1): 9-18.

1098 [30] Chang L, Karin M. Mammalian MAP kinase signalling cascades. *Nature* 2001; 410(6824): 37-40.

1099 [31] Wagner EF, Nebreda AR. Signal integration by JNK and p38 MAPK pathways in cancer
1100 development. *Nat Rev Cancer* 2009; 9(8): 537-49.

1101 [32] Alfadda AA, Sallam RM. Reactive oxygen species in health and disease. *J Biomed Biotechnol* 2012;
1102 2012: 936486.

1103 [33] Klaunig JE, Kamendulis LM, Hocevar BA. Oxidative stress and oxidative damage in carcinogenesis.
1104 *Toxicol Pathol* 2010; 38(1): 96-109.

1105 [34] Waris G, Ahsan H. Reactive oxygen species: role in the development of cancer and various chronic
1106 conditions. *J Carcinog* 2006; 5: 14.

1107 [35] Bridge G, Rashid S, Martin SA. DNA mismatch repair and oxidative DNA damage: implications for
1108 cancer biology and treatment. *Cancers (Basel)* 2014; 6(3): 1597-614.

1109 [36] Hernandez-Garcia D, Castro-Obregon S, Gomez-Lopez S, Valencia C, Covarrubias L. Cell death
1110 activation during cavitation of embryoid bodies is mediated by hydrogen peroxide. *Exp Cell Res* 2008;
1111 314(10): 2090-9.

1112 [37] Dewaele M, Maes H, Agostinis P. ROS-mediated mechanisms of autophagy stimulation and their
1113 relevance in cancer therapy. *Autophagy* 2010; 6(7): 838-54.

1114 [38] Scherz-Shouval R, Shvets E, Fass E, Shorer H, Gil L, Elazar Z. Reactive oxygen species are essential
1115 for autophagy and specifically regulate the activity of Atg4. *EMBO J* 2007; 26(7): 1749-60.

1116 [39] Kawanishi S, Oikawa S. Mechanism of telomere shortening by oxidative stress. *Ann N Y Acad Sci*
1117 2004; 1019: 278-84.

1118 [40] Petersen S, Saretzki G, von Zglinicki T. Preferential accumulation of single-stranded regions in
1119 telomeres of human fibroblasts. *Exp Cell Res* 1998; 239(1): 152-60.

1120 [41] Indran IR, Hande MP, Pervaiz S. hTERT overexpression alleviates intracellular ROS production,
1121 improves mitochondrial function, and inhibits ROS-mediated apoptosis in cancer cells. *Cancer Res*
1122 2011; 71(1): 266-76.

1123 [42] Sahin E, Depinho RA. Linking functional decline of telomeres, mitochondria and stem cells during
1124 ageing. *Nature* 2010; 464(7288): 520-8.

1125 [43] Chen L, Liu L, Yin J, Luo Y, Huang S. Hydrogen peroxide-induced neuronal apoptosis is associated
1126 with inhibition of protein phosphatase 2A and 5, leading to activation of MAPK pathway. *Int J Biochem*
1127 *Cell Biol* 2009; 41(6): 1284-95.

1128 [44] Funato Y, Michiue T, Asashima M, Miki H. The thioredoxin-related redox-regulating protein
1129 nucleoredoxin inhibits Wnt-beta-catenin signalling through dishevelled. *Nat Cell Biol* 2006; 8(5): 501-
1130 8.

1131 [45] Korswagen HC. Regulation of the Wnt/beta-catenin pathway by redox signaling. *Dev Cell* 2006;
1132 10(6): 687-8.

1133 [46] Kajla S, Mondol AS, Nagasawa A, *et al.* A crucial role for Nox 1 in redox-dependent regulation of
1134 Wnt-beta-catenin signaling. *FASEB J* 2012; 26(5): 2049-59.

1135 [47] Wen JW, Hwang JT, Kelly GM. Reactive oxygen species and Wnt signalling crosstalk patterns mouse
1136 extraembryonic endoderm. *Cell Signal* 2012; 24(12): 2337-48.

1137 [48] Sandieson L, Hwang JT, Kelly GM. Redox regulation of canonical Wnt signaling affects
1138 extraembryonic endoderm formation. *Stem Cells Dev* 2014; 23(10): 1037-49.

1139 [49] Ushio-Fukai M, Rehman J. Redox and metabolic regulation of stem/progenitor cells and their
1140 niche. *Antioxid Redox Signal* 2014; 21(11): 1587-90.

1141 [50] Finkel T. Signal transduction by mitochondrial oxidants. *J Biol Chem* 2012; 287(7): 4434-40.

1142 [51] Wang K, Zhang T, Dong Q, Nice EC, Huang C, Wei Y. Redox homeostasis: the linchpin in stem cell
1143 self-renewal and differentiation. *Cell Death Dis* 2013; 4: e537.

1144 [52] Nair AR, Degheselle O, Smeets K, Van Kerkhove E, Cuypers A. Cadmium-Induced Pathologies:
1145 Where Is the Oxidative Balance Lost (or Not)? *Int J Mol Sci* 2013; 14(3): 6116-43.

1146 [53] Fehrer C, Brunauer R, Laschober G, *et al.* Reduced oxygen tension attenuates differentiation
1147 capacity of human mesenchymal stem cells and prolongs their lifespan. *Aging Cell* 2007; 6(6): 745-57.

1148 [54] Sanchez Alvarado A. Cellular hyperproliferation and cancer as evolutionary variables. *Curr Biol*
1149 2012; 22(17): R772-8.

1150 [55] Sudhakar A. History of Cancer, Ancient and Modern Treatment Methods. *J Cancer Sci Ther* 2009;
1151 1(2): 1-4.

1152 [56] Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; 144(5): 646-74.

1153 [57] Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: how
1154 are they linked? *Free Radic Biol Med* 2010; 49(11): 1603-16.

1155 [58] Sedelnikova OA, Redon CE, Dickey JS, Nakamura AJ, Georgakilas AG, Bonner WM. Role of
1156 oxidatively induced DNA lesions in human pathogenesis. *Mutation Research/Reviews in Mutation*
1157 *Research* 2010; 704(1-3): 152-9.

1158 [59] Goodsell DS. The molecular perspective: the ras oncogene. *Oncologist* 1999; 4(3): 263-4.

1159 [60] Ozaki T, Nakagawara A. Role of p53 in Cell Death and Human Cancers. *Cancers (Basel)* 2011; 3(1):
1160 994-1013.

1161 [61] Pomerantz JH, Blau HM. Tumor suppressors: enhancers or suppressors of regeneration?
1162 *Development* 2013; 140(12): 2502-12.

1163 [62] Rivlin N, Brosh R, Oren M, Rotter V. Mutations in the p53 Tumor Suppressor Gene: Important
1164 Milestones at the Various Steps of Tumorigenesis. *Genes Cancer* 2011; 2(4): 466-74.

1165 [63] Olivier M, Hollstein M, Hainaut P. TP53 Mutations in Human Cancers: Origins, Consequences, and
1166 Clinical Use. *Cold Spring Harb Perspect Biol* 2010; 2(1).

1167 [64] Raatikainen S, Aaltomaa S, Kärjä V, Soini Y. Increased nuclear factor erythroid 2-related factor 2
1168 expression predicts worse prognosis of prostate cancer patients treated with radical prostatectomy.
1169 *Hum Pathol*; (0).

1170 [65] Wang W, He YF, Sun QK, *et al.* Hypoxia-inducible factor 1alpha in breast cancer prognosis. *Clin*
1171 *Chim Acta* 2014; 428: 32-7.

1172 [66] Huang M, Chen Q, Xiao J, *et al.* Overexpression of hypoxia-inducible factor-1alpha is a predictor
1173 of poor prognosis in cervical cancer: a clinicopathologic study and a meta-analysis. *Int J Gynecol Cancer*
1174 2014; 24(6): 1054-64.

1175 [67] Ye LY, Zhang Q, Bai XL, Pankaj P, Hu QD, Liang TB. Hypoxia-inducible factor 1alpha expression and
1176 its clinical significance in pancreatic cancer: A meta-analysis. *Pancreatology* 2014; 14(5): 391-7.

1177 [68] Liou GY, Storz P. Reactive oxygen species in cancer. *Free Radic Res* 2010; 44(5): 479-96.

1178 [69] Fiaschi T, Chiarugi P. Oxidative stress, tumor microenvironment, and metabolic reprogramming: a
1179 diabolic liaison. *Int J Cell Biol* 2012; 2012: 762825.

1180 [70] Pani G, Galeotti T, Chiarugi P. Metastasis: cancer cell's escape from oxidative stress. *Cancer and*
1181 *Metastasis Reviews* 2010; 29(2): 351-78.

1182 [71] Bell EL, Klimova TA, Eisenbart J, Schumacker PT, Chandel NS. Mitochondrial reactive oxygen
1183 species trigger hypoxia-inducible factor-dependent extension of the replicative life span during
1184 hypoxia. *Mol Cell Biol* 2007; 27(16): 5737-45.

1185 [72] Xia C, Meng Q, Liu LZ, Rojanasakul Y, Wang XR, Jiang BH. Reactive oxygen species regulate
1186 angiogenesis and tumor growth through vascular endothelial growth factor. *Cancer Res* 2007; 67(22):
1187 10823-30.

1188 [73] Liu C, Huang H, Wang C, Kong Y, Zhang H, Zhang H. Association between OGG1 gene single
1189 nucleotide polymorphisms and risk of pancreatic cancer in Chinese. *Med Oncol* 2014; 31(7): 40.

1190 [74] Diaz B, Shani G, Pass I, Anderson D, Quintavalle M, Courtneidge SA. Tks5-dependent, nox-
1191 mediated generation of reactive oxygen species is necessary for invadopodia formation. *Sci Signal*
1192 2009; 2(88): ra53.

1193 [75] Costa A, Scholer-Dahirel A, Mechta-Grigoriou F. The role of reactive oxygen species and
1194 metabolism on cancer cells and their microenvironment. *Semin Cancer Biol* 2014; 25: 23-32.

1195 [76] Roessner A, Kuester D, Malfertheiner P, Schneider-Stock R. Oxidative stress in ulcerative colitis-
1196 associated carcinogenesis. *Pathology - Research and Practice* 2008; 204(7): 511-24.

1197 [77] Warburg O. On the origin of cancer cells. *Science* 1956; 123(3191): 309-14.

1198 [78] Pavlides S, Whitaker-Menezes D, Castello-Cros R, *et al.* The reverse Warburg effect: aerobic
1199 glycolysis in cancer associated fibroblasts and the tumor stroma. *Cell Cycle* 2009; 8(23): 3984-4001.

1200 [79] Martinez-Outschoorn UE, Balliet RM, Rivadeneira DB, *et al.* Oxidative stress in cancer associated
1201 fibroblasts drives tumor-stroma co-evolution: A new paradigm for understanding tumor metabolism,
1202 the field effect and genomic instability in cancer cells. *Cell Cycle* 2010; 9(16): 3256-76.

1203 [80] Martinez-Outschoorn UE, Trimmer C, Lin Z, *et al.* Autophagy in cancer associated fibroblasts
1204 promotes tumor cell survival: Role of hypoxia, HIF1 induction and NFkappaB activation in the tumor
1205 stromal microenvironment. *Cell Cycle* 2010; 9(17): 3515-33.

1206 [81] Pavlides S, Vera I, Gandara R, *et al.* Warburg meets autophagy: cancer-associated fibroblasts
1207 accelerate tumor growth and metastasis via oxidative stress, mitophagy, and aerobic glycolysis.
1208 *Antioxid Redox Signal* 2012; 16(11): 1264-84.

1209 [82] Yang ZJ, Chee CE, Huang S, Sinicrope FA. The role of autophagy in cancer: therapeutic implications.
1210 *Mol Cancer Ther* 2011; 10(9): 1533-41.

1211 [83] Donatelli SS, Zhou JM, Gilvary DL, *et al.* TGF-beta-inducible microRNA-183 silences tumor-
1212 associated natural killer cells. *Proc Natl Acad Sci U S A* 2014; 111(11): 4203-8.

1213 [84] Barcellos-Hoff MH, Dix TA. Redox-mediated activation of latent transforming growth factor-beta
1214 1. *Mol Endocrinol* 1996; 10(9): 1077-83.

1215 [85] Gupta SC, Hevia D, Patchva S, Park B, Koh W, Aggarwal BB. Upsides and Downsides of Reactive
1216 Oxygen Species for Cancer: The Roles of Reactive Oxygen Species in Tumorigenesis, Prevention, and
1217 Therapy. *Antioxid Redox Signal* 2011; 16(11): 1295-322.

1218 [86] Cheung EC, Athineos D, Lee P, *et al.* TIGAR is required for efficient intestinal regeneration and
1219 tumorigenesis. *Dev Cell* 2013; 25(5): 463-77.

1220 [87] Yang W, Zou L, Huang C, Lei Y. Redox Regulation of Cancer Metastasis: Molecular Signaling and
1221 Therapeutic Opportunities. *Drug Dev Res* 2014; 75(5): 331-41.

1222 [88] Ushio-Fukai M, Nakamura Y. Reactive oxygen species and angiogenesis: NADPH oxidase as target
1223 for cancer therapy. *Cancer Lett* 2008; 266(1): 37-52.

1224 [89] Khandrika L, Kumar B, Koul S, Maroni P, Koul HK. Oxidative stress in prostate cancer. *Cancer Lett*
1225 2009; 282(2): 125-36.

1226 [90] Clerkin JS, Naughton R, Quiney C, Cotter TG. Mechanisms of ROS modulated cell survival during
1227 carcinogenesis. *Cancer Lett* 2008; 266(1): 30-6.

1228 [91] Vafa O, Wade M, Kern S, *et al.* c-Myc can induce DNA damage, increase reactive oxygen species,
1229 and mitigate p53 function: a mechanism for oncogene-induced genetic instability. *Mol Cell* 2002; 9(5):
1230 1031-44.

1231 [92] Shinohara M, Shang W-H, Kubodera M, *et al.* Nox1 Redox Signaling Mediates Oncogenic Ras-
1232 induced Disruption of Stress Fibers and Focal Adhesions by Down-regulating Rho. *J Biol Chem* 2007;
1233 282(24): 17640-8.

1234 [93] Kopnin PB, Agapova LS, Kopnin BP, Chumakov PM. Repression of sestrin family genes contributes
1235 to oncogenic Ras-induced reactive oxygen species up-regulation and genetic instability. *Cancer Res*
1236 2007; 67(10): 4671-8.

1237 [94] Sablina AA, Budanov AV, Ilyinskaya GV, Agapova LS, Kravchenko JE, Chumakov PM. The antioxidant
1238 function of the p53 tumor suppressor. *Nat Med* 2005; 11(12): 1306-13.

1239 [95] Trachootham D, Chen G, Zhang W, *et al.* Loss of p53 in stromal fibroblasts promotes epithelial cell
1240 invasion through redox-mediated ICAM1 signal. *Free Radical Biol Med* 2013; 58: 1-13.

1241 [96] Greaves LC, Reeve AK, Taylor RW, Turnbull DM. Mitochondrial DNA and disease. *The Journal of*
1242 *Pathology* 2012; 226(2): 274-86.

1243 [97] Piruat JI, Millan-Ucles A. Genetically modeled mice with mutations in mitochondrial metabolic
1244 enzymes for the study of cancer. *Front Oncol* 2014; 4: 200.

1245 [98] Jiao F, Liu Y, Qu Y, *et al.* Studies on anti-tumor and antimetastatic activities of fullereneol in a mouse
1246 breast cancer model. *Carbon* 2010; 48(8): 2231-43.

1247 [99] Ilmer M, Vykoukal J, Recio Boiles A, Coleman M, Alt E. Two sides of the same coin: stem cells in
1248 cancer and regenerative medicine. *FASEB J* 2014; 28(7): 2748-61.

1249 [100] Heddleston JM, Li Z, Lathia JD, Bao S, Hjelmeland AB, Rich JN. Hypoxia inducible factors in cancer
1250 stem cells. *Br J Cancer* 2010; 102(5): 789-95.

1251 [101] Cairns RA, Harris IS, Mak TW. Regulation of cancer cell metabolism. *Nat Rev Cancer* 2011; 11(2):
1252 85-95.

1253 [102] Hileman EO, Liu J, Albitar M, Keating MJ, Huang P. Intrinsic oxidative stress in cancer cells: a
1254 biochemical basis for therapeutic selectivity. *Cancer Chemother Pharmacol* 2004; 53(3): 209-19.

1255 [103] Carew JS, Zhou Y, Albitar M, Carew JD, Keating MJ, Huang P. Mitochondrial DNA mutations in
1256 primary leukemia cells after chemotherapy: clinical significance and therapeutic implications.
1257 *Leukemia* 2003; 17(8): 1437-47.

1258 [104] Acharya A, Das I, Chandhok D, Saha T. Redox regulation in cancer: a double-edged sword with
1259 therapeutic potential. *Oxid Med Cell Longev* 2010; 3(1): 23-34.

1260 [105] Cui X. Reactive Oxygen Species: The Achilles' Heel of Cancer Cells? *Antioxidants & Redox Signaling*
1261 2012; 16(11): 1212-4.

1262 [106] Nagano O, Okazaki S, Saya H. Redox regulation in stem-like cancer cells by CD44 variant isoforms.
1263 *Oncogene* 2013; 32(44): 5191-8.

1264 [107] Ishimoto T, Nagano O, Yae T, *et al.* CD44 Variant Regulates Redox Status in Cancer Cells by
1265 Stabilizing the xCT Subunit of System xc⁻ and Thereby Promotes Tumor Growth. *Cancer Cell* 2011;
1266 19(3): 387-400.

1267 [108] Oviedo NJ, Beane WS. Regeneration: The origin of cancer or a possible cure? *Semin Cell Dev Biol*
1268 2009; 20(5): 557-64.

1269 [109] Seifert AW, Voss SR. Revisiting the relationship between regenerative ability and aging. *BMC Biol*
1270 2013; 11: 2.

1271 [110] Galliot B. Injury-induced asymmetric cell death as a driving force for head regeneration in Hydra.
1272 *Dev Genes Evol* 2013; 223(1-2): 39-52.

1273 [111] Endo T, Bryant SV, Gardiner DM. A stepwise model system for limb regeneration. *Dev Biol* 2004;
1274 270(1): 135-45.

1275 [112] Mathew LK, Sengupta S, Kawakami A, *et al.* Unraveling tissue regeneration pathways using
1276 chemical genetics. *J Biol Chem* 2007; 282(48): 35202-10.

1277 [113] Brockes JP, Kumar A. Comparative aspects of animal regeneration. *Annu Rev Cell Dev Biol* 2008;
1278 24: 525-49.

1279 [114] Kennedy KA, Sandiford SD, Skerjanc IS, Li SS. Reactive oxygen species and the neuronal fate. *Cell*
1280 *Mol Life Sci* 2012; 69(2): 215-21.

1281 [115] Bedard K, Krause KH. The NOX family of ROS-generating NADPH oxidases: physiology and
1282 pathophysiology. *Physiol Rev* 2007; 87(1): 245-313.

1283 [116] Suzukawa K, Miura K, Mitsushita J, *et al.* Nerve growth factor-induced neuronal differentiation
1284 requires generation of Rac1-regulated reactive oxygen species. *J Biol Chem* 2000; 275(18): 13175-8.

1285 [117] Kennedy KA, Ostrakhovitch EA, Sandiford SD, *et al.* Mammalian numb-interacting protein 1/dual
1286 oxidase maturation factor 1 directs neuronal fate in stem cells. *J Biol Chem* 2010; 285(23): 17974-85.

1287 [118] Min JY, Park MH, Park MK, *et al.* Staurosporin induces neurite outgrowth through ROS generation
1288 in HN33 hippocampal cell lines. *J Neural Transm Gen Sect* 2006; 113(11): 1821-6.

1289 [119] Gotenstein JR, Swale RE, Fukuda T, *et al.* The *C. elegans* peroxidase PNX-2 is essential for
1290 embryonic morphogenesis and inhibits adult axon regeneration. *Development* 2010; 137(21): 3603-
1291 13.

1292 [120] Rieger S, Sagasti A. Hydrogen peroxide promotes injury-induced peripheral sensory axon
1293 regeneration in the zebrafish skin. *PLoS Biol* 2011; 9(5): e1000621.

1294 [121] Gauron C, Rampon C, Bouzaffour M, *et al.* Sustained production of ROS triggers compensatory
1295 proliferation and is required for regeneration to proceed. *Sci Rep* 2013; 3: 2084.

1296 [122] Love NR, Chen Y, Ishibashi S, *et al.* Amputation-induced reactive oxygen species are required for
1297 successful *Xenopus* tadpole tail regeneration. *Nat Cell Biol* 2013; 15(2): 222-8.

1298 [123] Niethammer P, Grabher C, Look AT, Mitchison TJ. A tissue-scale gradient of hydrogen peroxide
1299 mediates rapid wound detection in zebrafish. *Nature* 2009; 459(7249): 996-9.

1300 [124] Adell T, Cebrià F, Saló E. Gradients in Planarian Regeneration and Homeostasis. *Cold Spring Harb*
1301 *Perspect Biol* 2010; 2(1).

1302 [125] Nejak-Bowen KN, Monga SP. Beta-catenin signaling, liver regeneration and hepatocellular
1303 cancer: sorting the good from the bad. *Semin Cancer Biol* 2011; 21(1): 44-58.

1304 [126] Tasaki J, Shibata N, Sakurai T, Agata K, Umesono Y. Role of c-Jun N-terminal kinase activation in
1305 blastema formation during planarian regeneration. *Dev Growth Differ* 2011; 53(3): 389-400.

1306 [127] Tasaki J, Shibata N, Nishimura O, *et al.* ERK signaling controls blastema cell differentiation during
1307 planarian regeneration. *Development* 2011; 138(12): 2417-27.

1308 [128] Umesono Y, Tasaki J, Nishimura Y, *et al.* The molecular logic for planarian regeneration along the
1309 anterior-posterior axis. *Nature* 2013; 500(7460): 73-6.

1310 [129] Agata K, Tasaki J, Nakajima E, Umesono Y. Recent identification of an ERK signal gradient
1311 governing planarian regeneration. *Zoology (Jena)* 2014; 117(3): 161-2.

1312 [130] King RS, Newmark PA. The cell biology of regeneration. *J Cell Biol* 2012; 196(5): 553-62.

1313 [131] Harsum S, Clarke JD, Martin P. A reciprocal relationship between cutaneous nerves and repairing
1314 skin wounds in the developing chick embryo. *Dev Biol* 2001; 238(1): 27-39.

1315 [132] Cebria F, Newmark PA. Morphogenesis defects are associated with abnormal nervous system
1316 regeneration following roboA RNAi in planarians. *Development* 2007; 134(5): 833-7.

1317 [133] Oviedo N, Morokuma J, Walentek P, *et al.* Long-range neural and gap junction protein-mediated
1318 cues control polarity during planarian regeneration. *Dev Biol* 2010; 339: 188 - 99.

1319 [134] Nix P, Hisamoto N, Matsumoto K, Bastiani M. Axon regeneration requires coordinate activation
1320 of p38 and JNK MAPK pathways. *Proc Natl Acad Sci U S A* 2011; 108(26): 10738-43.

1321 [135] Hammarlund M, Nix P, Hauth L, Jorgensen EM, Bastiani M. Axon regeneration requires a
1322 conserved MAP kinase pathway. *Science* 2009; 323(5915): 802-6.

1323 [136] Ling S, Chang X, Schultz L, *et al.* An EGFR-ERK-SOX9 Signaling Cascade Links Urothelial
1324 Development and Regeneration to Cancer. *Cancer Res* 2011; 71(11): 3812-21.

1325 [137] Levin M. Morphogenetic fields in embryogenesis, regeneration, and cancer: non-local control of
1326 complex patterning. *BioSyst* 2012; 109(3): 243-61.

1327 [138] Riss J, Khanna C, Koo S, *et al.* Cancers as wounds that do not heal: differences and similarities
1328 between renal regeneration/repair and renal cell carcinoma. *Cancer Res* 2006; 66(14): 7216-24.

1329 [139] Tögel F, Westenfelder C. The role of multipotent marrow stromal cells (MSCs) in tissue
1330 regeneration. *Organogenesis* 2011; 7(2): 96-100.

1331 [140] Bergfeld SA, DeClerck YA. Bone marrow-derived mesenchymal stem cells and the tumor
1332 microenvironment. *Cancer and Metastasis Reviews* 2010; 29(2): 249-61.

1333 [141] Jiang Z, Zacksenhaus E, Gallie BL, Phillips RA. The retinoblastoma gene family is differentially
1334 expressed during embryogenesis. *Oncogene* 1997; 14(15): 1789-97.

1335 [142] Clarke AR, Maandag ER, van Roon M, *et al.* Requirement for a functional Rb-1 gene in murine
1336 development. *Nature* 1992; 359(6393): 328-30.

1337 [143] Yun MH, Gates PB, Brockes JP. Regulation of p53 is critical for vertebrate limb regeneration.
1338 *Proceedings of the National Academy of Sciences* 2013; 110(43): 17392-7.

1339 [144] Wells BS, Johnston LA. Maintenance of imaginal disc plasticity and regenerative potential in
1340 *Drosophila* by p53. *Dev Biol* 2012; 361(2): 263-76.

1341 [145] Di Giovanni S, Rathore K. p53-dependent pathways in neurite outgrowth and axonal
1342 regeneration. *Cell and Tissue Research* 2012; 349(1): 87-95.

1343 [146] Kurinna S, Stratton SA, Coban Z, *et al.* p53 regulates a mitotic transcription program and
1344 determines ploidy in normal mouse liver. *Hepatology* 2013; 57(5): 2004-13.

1345 [147] Hollander MC, Blumenthal GM, Dennis PA. PTEN loss in the continuum of common cancers, rare
1346 syndromes and mouse models. *Nat Rev Cancer* 2011; 11(4): 289-301.

1347 [148] Alimonti A, Carracedo A, Clohessy JG, *et al.* Subtle variations in Pten dose determine cancer
1348 susceptibility. *Nat Genet* 2010; 42(5): 454-8.

1349 [149] Park KK, Liu K, Hu Y, Kanter JL, He Z. PTEN/mTOR and axon regeneration. *Exp Neurol* 2010; 223(1):
1350 45-50.

1351 [150] Zeng N, Yang K-T, Bayan J-A, *et al.* PTEN controls β -cell regeneration in aged mice by regulating
1352 cell cycle inhibitor p16ink4a. *Aging Cell* 2013; 12(6): 1000-11.

1353 [151] Oviedo NJ, Pearson BJ, Levin M, Alvarado AS. Planarian PTEN homologs regulate stem cells and
1354 regeneration through TOR signaling. *Disease Models & Mechanisms* 2008; 1(2-3): 131-43.

1355 [152] Zhou D, Zhang Y, Wu H, *et al.* Mst1 and Mst2 protein kinases restrain intestinal stem cell
1356 proliferation and colonic tumorigenesis by inhibition of Yes-associated protein (Yap) overabundance.
1357 *Proceedings of the National Academy of Sciences* 2011; 108(49): E1312–E20.

1358 [153] Cai J, Zhang N, Zheng Y, de Wilde RF, Maitra A, Pan D. The Hippo signaling pathway restricts the
1359 oncogenic potential of an intestinal regeneration program. *Genes & Development* 2010; 24(21): 2383-
1360 8.

1361 [154] Demircan T, Berezikov E. The Hippo Pathway Regulates Stem Cells During Homeostasis and
1362 Regeneration of the Flatworm *Macrostomum Lignano*. *Stem Cells and Development* 2013; 22(15):
1363 2174-85.

1364 [155] Fraguas S, Barberan S, Cebria F. EGFR signaling regulates cell proliferation, differentiation and
1365 morphogenesis during planarian regeneration and homeostasis. *Dev Biol* 2011; 354(1): 87-101.

1366 [156] Normanno N, De Luca A, Bianco C, *et al.* Epidermal growth factor receptor (EGFR) signaling in
1367 cancer. *Gene* 2006; 366(1): 2-16.

1368 [157] Maemondo M, Inoue A, Kobayashi K, *et al.* Gefitinib or Chemotherapy for Non–Small-Cell Lung
1369 Cancer with Mutated EGFR. *New England Journal of Medicine* 2010; 362(25): 2380-8.

1370 [158] Nautiyal J, Majumder P, Patel BB, Lee FY, Majumdar APN. Src inhibitor dasatinib inhibits growth
1371 of breast cancer cells by modulating EGFR signaling. *Cancer Letters* 2009; 283(2): 143-51.

1372 [159] Jiang H, Grenley MO, Bravo M-J, Blumhagen RZ, Edgar BA. EGFR/Ras/MAPK Signaling Mediates
1373 Adult Midgut Epithelial Homeostasis and Regeneration in *Drosophila*. *Cell Stem Cell* 2011; 8(1): 84-95.

1374 [160] Seki E, Kondo Y, Iimuro Y, *et al.* Demonstration of cooperative contribution of MET- and EGFR-
1375 mediated STAT3 phosphorylation to liver regeneration by exogenous suppressor of cytokine signalings.
1376 *Journal of Hepatology* 2008; 48(2): 237-45.

1377 [161] Polakis P. Wnt signaling in cancer. *Cold Spring Harb Perspect Biol* 2012; 4(5).

1378 [162] Nejak-Bowen KN, Monga SPS. Beta-catenin signaling, liver regeneration and hepatocellular
1379 cancer: Sorting the good from the bad. *Semin Cancer Biol* 2011; 21(1): 44-58.

1380 [163] White BD, Chien AJ, Dawson DW. Dysregulation of Wnt/beta-catenin signaling in gastrointestinal
1381 cancers. *Gastroenterology* 2012; 142(2): 219-32.

1382 [164] Laplante M, Sabatini DM. mTOR signaling in growth control and disease. *Cell* 2012; 149(2): 274-
1383 93.

1384 [165] May C, Sphyris N, Evans K, Werden S, Guo W, Mani S. Epithelial-mesenchymal transition and
1385 cancer stem cells: a dangerously dynamic duo in breast cancer progression. *Breast Cancer Research*
1386 2011; 13(1): 202.

1387 [166] Micalizzi D, Farabaugh S, Ford H. Epithelial-Mesenchymal Transition in Cancer: Parallels Between
1388 Normal Development and Tumor Progression. *Journal of Mammary Gland Biology and Neoplasia* 2010;
1389 15(2): 117-34.

1390 [167] Zhou B, Pu WT. Epicardial epithelial-to-mesenchymal transition in injured heart. *Journal of*
1391 *Cellular and Molecular Medicine* 2011; 15(12): 2781-3.

1392 [168] Oviedo NJ, Levin M. smedinx-11 is a planarian stem cell gap junction gene required for
1393 regeneration and homeostasis. *Development* 2007; 134(17): 3121-31.

1394 [169] Salameh A, Dhein S. Pharmacology of Gap junctions. New pharmacological targets for treatment
1395 of arrhythmia, seizure and cancer? *Biochimica et Biophysica Acta (BBA) - Biomembranes* 2005; 1719(1–
1396 2): 36-58.

1397 [170] Mesnil M, Crespin S, Avanzo J-L, Zaidan-Dagli M-L. Defective gap junctional intercellular
1398 communication in the carcinogenic process. *Biochimica et Biophysica Acta (BBA) - Biomembranes*
1399 2005; 1719(1–2): 125-45.

1400 [171] McLachlan E, Shao Q, Laird DW. Connexins and gap junctions in mammary gland development
1401 and breast cancer progression. *Journal of Membrane Biology* 2007; 218(1-3): 107-21.

1402 [172] Adell T, Cebrià F, Saló E. Gradients in Planarian Regeneration and Homeostasis. *Cold Spring Harb*
1403 *Perspect Biol* 2010; 2(1).

1404 [173] Nogi T, Levin M. Characterization of innexin gene expression and functional roles of gap-
1405 junctional communication in planarian regeneration. *Developmental Biology* 2005; 287(2): 314-35.

1406 [174] Peiris TH, Hoyer KK, Oviedo NJ. Innate immune system and tissue regeneration in planarians: An
1407 area ripe for exploration. *Semin Immunol* 2014; 26(4): 295-302.

1408 [175] Allen JE, Sutherland TE. Host protective roles of type 2 immunity: Parasite killing and tissue repair,
1409 flip sides of the same coin. *Semin Immunol* 2014; 26(4): 329-40.

1410 [176] Godwin JW, Pinto AR, Rosenthal NA. Macrophages are required for adult salamander limb
1411 regeneration. *Proc Natl Acad Sci U S A* 2013; 110(23): 9415-20.

1412 [177] Godwin JW, Rosenthal N. Scar-free wound healing and regeneration in amphibians:
1413 immunological influences on regenerative success. *Differentiation* 2014; 87(1-2): 66-75.

1414 [178] Wenger Y, Buzgariu W, Reiter S, Galliot B. Injury-induced immune responses in Hydra. *Semin*
1415 *Immunol* 2014; 26(4): 277-94.

1416 [179] Eming SA, Hammerschmidt M, Krieg T, Roers A. Interrelation of immunity and tissue repair or
1417 regeneration. *Semin Cell Dev Biol* 2009; 20(5): 517-27.

1418 [180] Finkel T. Oxidant signals and oxidative stress. *Curr Opin Cell Biol* 2003; 15(2): 247-54.

1419 [181] Glasauer A, Chandel NS. Targeting antioxidants for cancer therapy. *Biochem Pharmacol* 2014.

1420 [182] Wondrak GT. Redox-Directed Cancer Therapeutics: Molecular Mechanisms and Opportunities.
1421 *Antioxid Redox Signal* 2009; 11(12): 3013-69.

1422 [183] Sreevalsan S, Safe S. Reactive oxygen species and colorectal cancer. *Curr Colorectal Cancer Rep*
1423 2013; 9(4): 350-7.

1424 [184] Fruehauf JP, Meyskens FL. Reactive Oxygen Species: A Breath of Life or Death? *Clinical Cancer*
1425 *Research* 2007; 13(3): 789-94.

1426 [185] Hagen H, Marzenell P, Jentzsch E, Wenz F, Veldwijk MR, Mokhir A. Aminoferrocene-Based
1427 Prodrugs Activated by Reactive Oxygen Species. *J Med Chem* 2012; 55(2): 924-34.

1428 [186] Sinha B, Mason R. Biotransformation of Hydrazine Derivatives in the Mechanism of Toxicity. *J*
1429 *Drug Metab Toxicol* 2014; 5(168): 2.

1430 [187] Weber JS, Samlowski WE, Gonzalez R, *et al.* A phase 1-2 study of imexon plus dacarbazine in
1431 patients with unresectable metastatic melanoma. *Cancer* 2010; 116(15): 3683-91.

1432 [188] Nagai M, Vo NH, Shin Ogawa L, *et al.* The oncology drug elesclomol selectively transports copper
1433 to the mitochondria to induce oxidative stress in cancer cells. *Free Radical Biology and Medicine* 2012;
1434 52(10): 2142-50.

1435 [189] Xie C-M, Chan WY, Yu S, Zhao J, Cheng CHK. Bufalin induces autophagy-mediated cell death in
1436 human colon cancer cells through reactive oxygen species generation and JNK activation. *Free Radical*
1437 *Biology and Medicine* 2011; 51(7): 1365-75.

1438 [190] Raj L, Ide T, Gurkar AU, *et al.* Selective killing of cancer cells by a small molecule targeting the
1439 stress response to ROS. *Nature* 2011; 475(7355): 231-4.

1440 [191] Liu JM, Pan F, Li L, *et al.* Piperlongumine selectively kills glioblastoma multiforme cells via reactive
1441 oxygen species accumulation dependent JNK and p38 activation. *Biochemical and Biophysical Research*
1442 *Communications* 2013; 437(1): 87-93.

1443 [192] Dhillon H, Chikara S, Reindl KM. Piperlongumine induces pancreatic cancer cell death by
1444 enhancing reactive oxygen species and DNA damage. *Toxicology Reports* 2014; 1(0): 309-18.

1445 [193] Golovine KV, Makhov PB, Teper E, *et al.* Piperlongumine induces rapid depletion of the androgen
1446 receptor in human prostate cancer cells. *The Prostate* 2013; 73(1): 23-30.

1447 [194] Jin H-O, Lee Y-H, Park J-A, *et al.* Piperlongumine induces cell death through ROS-mediated CHOP
1448 activation and potentiates TRAIL-induced cell death in breast cancer cells. *Journal of Cancer Research*
1449 *and Clinical Oncology* 2014: 1-8.

1450 [195] Yang F, Chen W-D, Deng R, *et al.* Hirsutanol A Induces Apoptosis and Autophagy via Reactive
1451 Oxygen Species Accumulation in Breast Cancer MCF-7 Cells. *Journal of Pharmacological Sciences* 2012;
1452 119(3): 214-20.

1453 [196] Yang F, Chen W-D, Deng R, *et al.* Hirsutanol A, a novel sesquiterpene compound from fungus
1454 *Chondrostereum* sp., induces apoptosis and inhibits tumor growth through mitochondrial-
1455 independent ROS production: Hirsutanol A inhibits tumor growth through ROS production. *Journal of*
1456 *Translational Medicine* 2013; 11(1): 32.

1457 [197] Pierre A-S, Minville-Walz M, Fèvre C, *et al.* Trans-10, cis-12 conjugated linoleic acid induced cell
1458 death in human colon cancer cells through reactive oxygen species-mediated ER stress. *Biochimica et*
1459 *Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* 2013; 1831(4): 759-68.

1460 [198] Bergamo P, Cocca E, Palumbo R, Gogliettino M, Rossi M, Palmieri G. RedOx Status, Proteasome
1461 and APEH: Insights into Anticancer Mechanisms of t10, c12-Conjugated Linoleic Acid Isomer on A375
1462 Melanoma Cells. *PloS one* 2013; 8(11): e80900.

1463 [199] Chen W, Feng L, Nie H, Zheng X. Andrographolide induces autophagic cell death in human liver
1464 cancer cells through cyclophilin D-mediated mitochondrial permeability transition pore.
1465 *Carcinogenesis* 2012; 33(11): 2190-8.

1466 [200] Lim JCW, Chan TK, Ng DSW, Sagineedu SR, Stanslas J, Wong WSF. Andrographolide and its
1467 analogues: versatile bioactive molecules for combating inflammation and cancer. *Clinical and*
1468 *Experimental Pharmacology and Physiology* 2012; 39(3): 300-10.

1469 [201] Yang S, Evens AM, Prachand S, *et al.* Mitochondrial-mediated apoptosis in lymphoma cells by the
1470 diterpenoid lactone andrographolide, the active component of *Andrographis paniculata*. *Clin Cancer*
1471 *Res* 2010; 16(19): 4755-68.

1472 [202] Yu JQ, Bao W, Lei JC. Emodin regulates apoptotic pathway in human liver cancer cells. *Phytother*
1473 *Res* 2013; 27(2): 251-7.

1474 [203] Wang W, Sun YP, Huang XZ, *et al.* Emodin enhances sensitivity of gallbladder cancer cells to
1475 platinum drugs via glutathion depletion and MRP1 downregulation. *Biochem Pharmacol* 2010; 79(8):
1476 1134-40.

1477 [204] Hou H, Li D, Cheng D, Li L, Liu Y, Zhou Y. Cellular Redox Status Regulates Emodin-Induced
1478 Radiosensitization of Nasopharyngeal Carcinoma Cells In Vitro and In Vivo. *Journal of Pharmaceutics*
1479 2013; 2013(doi: 10.1016/j.bcp.2014.07.017.).

1480 [205] Nishanth RP, Ramakrishna BS, Jyotsna RG, *et al.* C-Phycocyanin inhibits MDR1 through reactive
1481 oxygen species and cyclooxygenase-2 mediated pathways in human hepatocellular carcinoma cell line.
1482 *European Journal of Pharmacology* 2010; 649(1-3): 74-83.

1483 [206] Nakata W, Hayakawa Y, Nakagawa H, *et al.* Anti-tumor activity of the proteasome inhibitor
1484 bortezomib in gastric cancer. *Int J Oncol* 2011; 39(6): 1529-36.

1485 [207] Goel A, Aggarwal BB. Curcumin, the golden spice from Indian saffron, is a chemosensitizer and
1486 radiosensitizer for tumors and chemoprotector and radioprotector for normal organs. *Nutrition and*
1487 *cancer* 2010; 62(7): 919-30.

1488 [208] Chatterjee SJ, Pandey S. Chemo-resistant melanoma sensitized by tamoxifen to low dose
1489 curcumin treatment through induction of apoptosis and autophagy. *Cancer biology & therapy* 2011;
1490 11(2): 216-28.

1491 [209] Xu B, Guo X, Mathew S, *et al.* Triptolide simultaneously induces reactive oxygen species, inhibits
1492 NF-κB activity and sensitizes 5-fluorouracil in colorectal cancer cell lines. *Cancer Lett* 2010; 291(2): 200-
1493 8.

1494 [210] Salama S, Diaz-Arrastia C, Patel D, Botting S, Hatch S. 2-Methoxyestradiol, an Endogenous
1495 Estrogen Metabolite, Sensitizes Radioresistant MCF-7/FIR Breast Cancer Cells Through Multiple
1496 Mechanisms. *International Journal of Radiation Oncology*Biophysics* 2011; 80(1): 231-9.

1497 [211] Wang W, Sun Y, Li X, *et al.* Emodin potentiates the anticancer effect of cisplatin on gallbladder
1498 cancer cells through the generation of reactive oxygen species and the inhibition of survivin expression.
1499 *Oncol Rep* 2011; 26(5): 1143-8.

1500 [212] Hoshikawa H, Indo K, Mori T, Mori N. Enhancement of the radiation effects by D-allose in head
1501 and neck cancer cells. *Cancer Lett* 2011; 306(1): 60-6.

1502 [213] Yang ES, Choi MJ, Kim JH, Choi KS, Kwon TK. Withaferin A enhances radiation-induced apoptosis
1503 in Caki cells through induction of reactive oxygen species, Bcl-2 downregulation and Akt inhibition.
1504 Chemico-Biological Interactions 2011; 190(1): 9-15.

1505 [214] Chun YJ, Park IC, Park MJ, *et al.* Enhancement of radiation response in human cervical cancer
1506 cells in vitro and in vivo by arsenic trioxide (As₂O₃). FEBS Lett 2002; 519(1-3): 195-200.

1507 [215] Liu J, Du J, Zhang Y, *et al.* Suppression of the malignant phenotype in pancreatic cancer by
1508 overexpression of phospholipid hydroperoxide glutathione peroxidase. Hum Gene Ther 2006; 17(1):
1509 105-16.

1510 [216] Venkataraman S, Jiang X, Weydert C, *et al.* Manganese superoxide dismutase overexpression
1511 inhibits the growth of androgen-independent prostate cancer cells. Oncogene 2005; 24(1): 77-89.

1512 [217] Goh J, Enns L, Fatemie S, *et al.* Mitochondrial targeted catalase suppresses invasive breast cancer
1513 in mice. BMC Cancer 2011; 11(1): 191.

1514 [218] Hyoudou K, Nishikawa M, Kobayashi Y, Ikemura M, Yamashita F, Hashida M. SOD derivatives
1515 prevent metastatic tumor growth aggravated by tumor removal. Clin Exp Metastasis 2008; 25(5): 531-
1516 6.

1517 [219] Martín V, Herrera F, García-Santos G, Antolín I, Rodríguez-Blanco J, Rodríguez C. Signaling
1518 pathways involved in antioxidant control of glioma cell proliferation. Free Radical Biology and Medicine
1519 2007; 42(11): 1715-22.

1520 [220] Gianni D, Taulet N, Zhang H, *et al.* A Novel and Specific NADPH Oxidase-1 (Nox1) Small-Molecule
1521 Inhibitor Blocks the Formation of Functional Invadopodia in Human Colon Cancer Cells. ACS Chemical
1522 Biology 2010; 5(10): 981-93.

1523 [221] Brar SS, Corbin Z, Kennedy TP, *et al.* NOX5 NAD(P)H oxidase regulates growth and apoptosis in
1524 DU 145 prostate cancer cells. Am J Physiol Cell Physiol 2003; 285: C353-C69.

1525 [222] Mochizuki T, Furuta S, Mitsushita J, *et al.* Inhibition of NADPH oxidase 4 activates apoptosis via
1526 the AKT/apoptosis signal-regulating kinase 1 pathway in pancreatic cancer PANC-1 cells. Oncogene
1527 2006; 25(26): 3699-707.

1528 [223] Lemmo W. Potential interactions of prescription and over-the-counter medications having
1529 antioxidant capabilities with radiation and chemotherapy. Int J Cancer 2014: doi: 10.1002/ijc.29208

1530 [224] VOLLBRACHT C, SCHNEIDER B, LEENDERT V, WEISS G, AUERBACH L, BEUTH J. Intravenous Vitamin
1531 C Administration Improves Quality of Life in Breast Cancer Patients during Chemo-/Radiotherapy and
1532 Aftercare: Results of a Retrospective, Multicentre, Epidemiological Cohort Study in Germany. In Vivo
1533 2011; 25(6): 983-90.

1534 [225] Fuchs-Tarlovsky V. Role of antioxidants in cancer therapy. Nutrition 2013; 29(1): 15-21.

1535 [226] Li Y, Li X, Wong Y-S, *et al.* The reversal of cisplatin-induced nephrotoxicity by selenium
1536 nanoparticles functionalized with 11-mercapto-1-undecanol by inhibition of ROS-mediated apoptosis.
1537 Biomaterials 2011; 32(34): 9068-76.

1538 [227] Sayin VI, Ibrahim MX, Larsson E, Nilsson JA, Lindahl P, Bergo MO. Antioxidants Accelerate Lung
1539 Cancer Progression in Mice. Sci Transl Med 2014; 6(221).

1540 [228] Farnebo M, Bykov VJN, Wiman KG. The p53 tumor suppressor: A master regulator of diverse
1541 cellular processes and therapeutic target in cancer. Biochemical and Biophysical Research
1542 Communications 2010; 396(1): 85-9.

1543 [229] Tovar C, Graves B, Packman K, *et al.* MDM2 Small-Molecule Antagonist RG7112 Activates p53
1544 Signaling and Regresses Human Tumors in Preclinical Cancer Models. Cancer Research 2013; 73(8):
1545 2587-97.

1546 [230] Lu WJ, Lee NP, Kaul SC, *et al.* Mortalin-p53 interaction in cancer cells is stress dependent and
1547 constitutes a selective target for cancer therapy. Cell Death Differ 2011; 18(6): 1046-56.

1548 [231] Chen F, Wang W, El-Deiry WS. Current strategies to target p53 in cancer. Biochemical
1549 Pharmacology 2010; 80(5): 724-30.

1550 [232] D'Anneo A, Carlisi D, Lauricella M, *et al.* Parthenolide generates reactive oxygen species and
1551 autophagy in MDA-MB231 cells. A soluble parthenolide analogue inhibits tumour growth and
1552 metastasis in a xenograft model of breast cancer. Cell Death Dis 2013; 4: e891.

1553 [233] Xu Y, Fang F, Miriyala S, *et al.* KEAP1 Is a Redox Sensitive Target That Arbitrates the Opposing
1554 Radiosensitive Effects of Parthenolide in Normal and Cancer Cells. *Cancer Research* 2013; 73(14): 4406-
1555 17.

1556 [234] Liu L, Chen C, Gong W, *et al.* Epoxyeicosatrienoic Acids Attenuate Reactive Oxygen Species Level,
1557 Mitochondrial Dysfunction, Caspase Activation, and Apoptosis in Carcinoma Cells Treated with Arsenic
1558 Trioxide. *Journal of Pharmacology and Experimental Therapeutics* 2011; 339(2): 451-63.

1559 [235] Lu K-H, Lee H-J, Huang M-L, *et al.* Synergistic Apoptosis-Inducing Antileukemic Effects of Arsenic
1560 Trioxide and *Mucuna macrocarpa* Stem Extract in Human Leukemic Cells via a Reactive Oxygen Species-
1561 Dependent Mechanism. *Evid Based Complement Alternat Med* 2012; 2012: 921430.

1562 [236] Berndt C, Kurz T, Bannenberg S, Jacob R, Holmgren A, Brunk UT. Ascorbate and endocytosed
1563 Motexafin gadolinium induce lysosomal rupture. *Cancer Letters* 2011; 307(2): 119-23.

1564 [237] Marengo B, De Ciucis C, Verzola D, *et al.* Mechanisms of BSO (L-buthionine-S,R-sulfoximine)-
1565 induced cytotoxic effects in neuroblastoma. *Free Radical Biology and Medicine* 2008; 44(3): 474-82.

1566 [238] Syed Alwi S, Cavell B, Donlevy A, Packham G. Differential induction of apoptosis in human breast
1567 cancer cell lines by phenethyl isothiocyanate, a glutathione depleting agent. *Cell Stress Chaperones*
1568 2012; 17(5): 529-38.

1569 [239] Gupta P, Wright SE, Kim S-H, Srivastava SK. Phenethyl isothiocyanate: A comprehensive review
1570 of anti-cancer mechanisms. *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer* 2014; 1846(2):
1571 405-24.

1572 [240] Klauser E, Gulden M, Maser E, Seibert S, Seibert H. Additivity, antagonism, and synergy in arsenic
1573 trioxide-induced growth inhibition of C6 glioma cells: Effects of genistein, quercetin and buthionine-
1574 sulfoximine. *Food Chem Toxicol* 2014; 67: 212-21.

1575 [241] Ong P-S, Chan S-Y, Ho PC. Differential augmentative effects of buthionine sulfoximine and
1576 ascorbic acid in As₂O₃-induced ovarian cancer cell death: Oxidative stress-independent and-
1577 dependent cytotoxic potentiation. *Int J Oncol* 2011; 38(6): 1731-9.

1578 [242] Wu X, Zhu Y, Yan H, *et al.* Isothiocyanates induce oxidative stress and suppress the metastasis
1579 potential of human non-small cell lung cancer cells. *BMC Cancer* 2010; 10(1): 269.

1580 [243] Zhang L, Hao Q, Bao L, *et al.* Phenethyl isothiocyanate suppresses cervical carcinoma metastasis
1581 potential and its molecular mechanism. *Molecular medicine reports* 2014; 10(5): 2675-80.

1582 [244] Tuskorn O, Prawan A, Senggunprai L, Kukongviriyapan U, Kukongviriyapan V. Phenethyl
1583 isothiocyanate induces apoptosis of cholangiocarcinoma cells through interruption of glutathione and
1584 mitochondrial pathway. *Naunyn Schmiedeberg's Arch Pharmacol* 2013; 386(11): 1009-16.

1585 [245] Lee K, Briehl MM, Mazar AP, *et al.* The copper chelator ATN-224 induces peroxy-nitrite-dependent
1586 cell death in hematological malignancies. *Free Radical Biology and Medicine* 2013; 60: 157-67.

1587 [246] Juarez JC, Manuia M, Burnett ME, *et al.* Superoxide dismutase 1 (SOD1) is essential for H₂O₂-
1588 mediated oxidation and inactivation of phosphatases in growth factor signaling. *Proc Natl Acad Sci U S*
1589 *A* 2008; 105(20): 7147-52.

1590 [247] Wu X-J, Kassie F, Mersch-Sundermann V. The role of reactive oxygen species (ROS) production
1591 on diallyl disulfide (DADS) induced apoptosis and cell cycle arrest in human A549 lung carcinoma cells.
1592 *Mutat Res* 2005; 579(1-2): 115-24.

1593 [248] Yang JS, Chen GW, Hsia TC, *et al.* Diallyl disulfide induces apoptosis in human colon cancer cell
1594 line (COLO 205) through the induction of reactive oxygen species, endoplasmic reticulum stress,
1595 caspases cascade and mitochondrial-dependent pathways. *Food Chem Toxicol* 2009; 47(1): 171-9.

1596 [249] Kopetz S, Lesslie DP, Dallas NA, *et al.* Synergistic activity of the SRC family kinase inhibitor
1597 dasatinib and oxaliplatin in colon carcinoma cells is mediated by oxidative stress. *Cancer Res* 2009;
1598 69(9): 3842-9.

1599 [250] Kim H-S, Kim M-J, Kim EJ, Yang Y, Lee M-S, Lim J-S. Berberine-induced AMPK activation inhibits
1600 the metastatic potential of melanoma cells via reduction of ERK activity and COX-2 protein expression.
1601 *Biochem Pharmacol* 2012; 83(3): 385-94.

1602 [251] Adhikary A, Mohanty S, Lahiry L, Hossain DMS, Chakraborty S, Das T. Theaflavins retard human
1603 breast cancer cell migration by inhibiting NF- κ B via p53-ROS cross-talk. *FEBS Letters* 2010; 584(1): 7-
1604 14.

1605 [252] Lou J, Zhou X, Weng Q, *et al.* XQ2, a novel TPZ derivative, induced G2/M phase arrest and
1606 apoptosis under hypoxia in non-small cell lung cancer cells. *Bioscience, biotechnology, and*
1607 *biochemistry* 2010; 74(6): 1181-7.

1608 [253] Nishikawa M, Hashida M, Takakura Y. Catalase delivery for inhibiting ROS-mediated tissue injury
1609 and tumor metastasis. *Advanced Drug Delivery Reviews* 2009; 61(4): 319-26.

1610 [254] Hyoudou K, Nishikawa M, Ikemura M, *et al.* Prevention of pulmonary metastasis from
1611 subcutaneous tumors by binary system-based sustained delivery of catalase. *Journal of Controlled*
1612 *Release* 2009; 137(2): 110-5.

1613 [255] Mirzayans R, Andrais B, Scott A, Murray D. New insights into p53 signaling and cancer cell
1614 response to DNA damage: implications for cancer therapy. *J Biomed Biotechnol* 2012; 2012: 170325.

1615 [256] Shen L, Sun X, Fu Z, Yang G, Li J, Yao L. The Fundamental Role of the p53 Pathway in Tumor
1616 Metabolism and Its Implication in Tumor Therapy. *Clinical Cancer Research* 2012; 18(6): 1561-7.

1617 [257] Mirzayans R, Murray D. Pharmacological modulation of p53 function in cancer therapy. *Curr*
1618 *Signal Transduct Ther* 2008; 3(3): 183-94.

1619 [258] Orłowski RZ, Baldwin AS, Jr. NF-kappaB as a therapeutic target in cancer. *Trends Mol Med* 2002;
1620 8(8): 385-9.

1621 [259] Sun B, Karin M. The therapeutic value of targeting inflammation in gastrointestinal cancers.
1622 *Trends Pharmacol Sci* 2014; 35(7): 349-57.

1623 [260] Tornatore L, Sandomenico A, Raimondo D, *et al.* Cancer-Selective Targeting of the NF-kappaB
1624 Survival Pathway with GADD45beta/MKK7 Inhibitors. *Cancer Cell* 2014; 26(4): 495-508.

1625 [261] Lin Y, Bai L, Chen W, Xu S. The NF-kappaB activation pathways, emerging molecular targets for
1626 cancer prevention and therapy. *Expert Opin Ther Targets* 2010; 14(1): 45-55.

1627 [262] Yu X, Kensler T. Nrf2 as a target for cancer chemoprevention. *Mutat Res* 2005; 591(1-2): 93-102.

1628 [263] Jeong WS, Jun M, Kong AN. Nrf2: a potential molecular target for cancer chemoprevention by
1629 natural compounds. *Antioxid Redox Signal* 2006; 8(1-2): 99-106.

1630 [264] Zhang B, Wang XQ, Chen HY, Liu BH. Involvement of the Nrf2 Pathway in the Regulation of
1631 Pterostilbene-Induced Apoptosis in HeLa Cells via ER Stress. *J Pharmacol Sci* 2014; 126(3): 216-29.

1632 [265] Santarpia L, Lippman SM, El-Naggar AK. Targeting the MAPK-RAS-RAF signaling pathway in cancer
1633 therapy. *Expert Opin Ther Targets* 2012; 16(1): 103-19.

1634 [266] Miller CR, Oliver KE, Farley JH. MEK1/2 inhibitors in the treatment of gynecologic malignancies.
1635 *Gynecol Oncol* 2014; 133(1): 128-37.

1636 [267] Sebolt-Leopold JS, Herrera R. Targeting the mitogen-activated protein kinase cascade to treat
1637 cancer. *Nat Rev Cancer* 2004; 4(12): 937-47.

1638 [268] Goldkorn T, Balaban N, Matsukuma K, *et al.* EGF-Receptor phosphorylation and signaling are
1639 targeted by H2O2 redox stress. *American journal of respiratory cell and molecular biology* 1998; 19(5):
1640 786-98.

1641 [269] Roock WD, Vriendt VD, Normanno N, Ciardiello F, Tejpar S. KRAS, BRAF, PIK3CA, and PTEN
1642 mutations: implications for targeted therapies in metastatic colorectal cancer. *The Lancet Oncology*
1643 2011; 12(6): 594-603.

1644 [270] Baselga J. Why the Epidermal Growth Factor Receptor? The Rationale for Cancer Therapy.
1645 *Oncologist* 2002; 7(suppl 4): 2-8.

1646 [271] Lui VW, Grandis JR. EGFR-mediated cell cycle regulation. *Anticancer Res* 2002; 22(1A): 1-11.

1647 [272] Tan TC, Rahman R, Jaber-Hijazi F, *et al.* Telomere maintenance and telomerase activity are
1648 differentially regulated in asexual and sexual worms. *Proc Natl Acad Sci U S A* 2012; 109(11): 4209-14.

1649 [273] Ruden M, Puri N. Novel anticancer therapeutics targeting telomerase. *Cancer Treat Rev* 2013;
1650 39(5): 444-56.

1651 [274] Folini M. Editorial: targeting telomere maintenance mechanisms in cancer therapy. *Curr Pharm*
1652 *Des* 2014; 20(41): 6359-60.

1653 [275] Crees Z, Girard J, Rios Z, *et al.* Oligonucleotides and G-quadruplex Stabilizers: Targeting
1654 Telomeres and Telomerase in Cancer Therapy. *Curr Pharm Des* 2014; 20(41): 6422-37.

1655 [276] Lee P, Vousden KH, Cheung EC. TIGAR, TIGAR, burning bright. *Cancer Metab* 2014; 2(1): 1.

1656 [277] Rothwell PM, Fowkes FG, Belch JF, Ogawa H, Warlow CP, Meade TW. Effect of daily aspirin on
1657 long-term risk of death due to cancer: analysis of individual patient data from randomised trials. *Lancet*
1658 2011; 377(9759): 31-41.

1659 [278] Manda G. NMT, Neagu T.-M. Reactive Oxygen Species, Cancer and Anti-Cancer Therapies. *Curr*
1660 *Chem Biol* 2009; 3: 342-66.

1661 [279] Magnon C, Hall SJ, Lin J, *et al.* Autonomic nerve development contributes to prostate cancer
1662 progression. *Science* 2013; 341(6142): 1236361.

1663 [280] Barnett CM, Foote MC, Panizza B. Cutaneous head and neck malignancies with perineural spread
1664 to contralateral cranial nerves: an argument for extending postoperative radiotherapy volume. *J Clin*
1665 *Oncol* 2013; 31(18): e291-3.

1666 [281] Liebig C, Ayala G, Wilks J, *et al.* Perineural invasion is an independent predictor of outcome in
1667 colorectal cancer. *J Clin Oncol* 2009; 27(31): 5131-7.

1668 [282] Adriaenssens E, Vanhecke E, Saule P, *et al.* Nerve growth factor is a potential therapeutic target
1669 in breast cancer. *Cancer Res* 2008; 68(2): 346-51.

1670 [283] Mescher AL, Neff AW. Regenerative capacity and the developing immune system. *Adv Biochem*
1671 *Eng Biotechnol* 2005; 93: 39-66.

1672 [284] Yang SR, Rahman I, Trosko JE, Kang KS. Oxidative stress-induced biomarkers for stem cell-based
1673 chemical screening. *Prev Med* 2012; 54 Suppl: S42-9.

1674 [285] Borovski T, De Sousa E Melo F, Vermeulen L, Medema JP. Cancer Stem Cell Niche: The Place to
1675 Be. *Cancer Research* 2011; 71(3): 634-9.

1676 [286] Frank NY, Schatton T, Frank MH. The therapeutic promise of the cancer stem cell concept. *The*
1677 *Journal of clinical investigation* 2010; 120(1): 41-50.

1678 [287] Xu Y, Stamenkovic I, Yu Q. CD44 Attenuates Activation of the Hippo Signaling Pathway and Is a
1679 Prime Therapeutic Target for Glioblastoma. *Cancer Research* 2010; 70(6): 2455-64.

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