Chapter 10
Hedgehog Signaling and Cancer Treatment Resistance

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Introduction

The hedgehog (HH) signaling is critical for growth and differentiation during embryonic development and is required for the maintenance of somatic stem cells [1]. In adult cells, HH signaling has been implicated in the maintenance of homeostasis of stem or progenitor cells in a number of epithelial tissues, including intestinal epithelia [2]. HH signaling also contributes to physiologic processes of epithelial repair and regeneration after injury [3]. However, aberrant activation of HH signaling in tumors from a wide range of tissues may allow escape from regulatory mechanisms that cause the return to quiescence that normally follows regeneration [4, 5]. Activation of HH signaling by binding of secreted HH ligands (Sonic, Indian, and Desert) to the membrane receptor Patched (PTCH) results in the nuclear translocation of the Gli family and initiation of HH-related gene expression [1, 6], including genes controlling the cell cycle, cell adhesion, signal transduction, angiogenesis, and apoptosis [7]. Several studies have shown that unregulated progenitor cell proliferation induced by abnormal Sonic hedgehog (SHH) signaling has a role in carcinogenesis [5, 8, 9]. For example, small cell lung carcinoma (SCLC), one of the identified malignancies with HH overexpression, could block the growth by the treatment of smoothened (Smo) inhibitor cyclopamine or a monoclonal antibody blocking SHH [10].

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Cancer Treatment Resistance

Cancer treatment, including radiotherapy (RT) and chemotherapy are considered effective for many types of cancers for clinical benefit and improvement in survival. Chemoradiotherapy (CRT) has been recommended as a standard treatment strategy for solid cancers, such as those arising from head and neck [11], cervix uteri [12], lung [13], esophagus [14], pancreas [15], stomach [16], and rectum [17]. Although genotoxic agents are strongly linked to tumorigenesis, the cytotoxic effect of DNA damage is also a critical facet of cancer therapy. In fact, the majority of human tumors treated with genotoxic agents possibly induced secondary primary malignancies and facilitated therapy-resistant forms [18, 19]. The self-renewal of cancer stem cells (CSCs), DNA repair, drug trafficking system, and other factors expressed in cancer cells are considered to prevent the injury by the therapy. For example, radiation therapy enhanced HSP90 chaperone function, causing radio-resistant lung cancer cells [20]. CRT, concurrent combination of RT and chemotherapy, in cervical cancer has been evaluated that improved treatment outcomes and/or maximize efficiency in comparison with RT alone [21]. Articles reported that the causes for chemotherapy failure in cancer treatment reside in multiple levels, such as poor vascularization, hypoxia, intratumoral high interstitial fluid pressure, and phenotypic resistance to drug-induced toxicity through upregulated xenobiotic metabolism or DNA repair mechanisms and silencing of apoptotic pathways [22, 23]. Factors that have been demonstrated with cancer treatment resistance are listed as follows:

1. *Growth factors* (*GFs*). In the cervical cancer patients, tumors with higher GF were more sensitive to radiation than those with low GF [24]. For instance, radiation-activated epidermal growth factor receptor (EGFR) signaling in cancers, such as non-small cell lung cancer (NSCLC), leading to radioresistance by inducing cell proliferation and enhanced DNA repair [20].
2. *Protumorigenic signaling cascade*. Some tumors cause rapid proliferation phenomenon, accelerated proliferation, by stimulation of irradiation during the course of RT. Accelerated proliferation increases dose required to control tumor cells and is an important cause of acquiring tumor radioresistance [21]. The phosphatidylinositol-3-kinase (PI3K)–Akt pathway involved in several human cancers is frequently upregulated [25], which may cause a tumorigenic phenotype with increased cell proliferation, metastasis, and angiogenesis. Akt inhibitors may significantly reduce viability of certain CSCs [26] and sensitize them to chemotherapeutic agents [27]. Inhibition of the Akt pathway further causes delayed repair of ionizing radiation (IR)-induced DNA double-strand breaks (DSB) formation and radiosensitization [28], indicating that the activation of Akt signaling may underlie at least some cases of radiation resistance [29].
3. *Hypoxia*. Research on an in vivo solid tumor demonstrated that contained a certain proportion of hypoxic fractions [30]. The existence of hypoxic cells is well recognized as one of the major factors causing radiation resistance which possibly results in local failure after RT [31].

4. *P53 and the factors relating apoptosis*. Apoptosis is an active mode of cell death which occurs in response to DNA damage by ionizing radiation, ultraviolet irradiation, and certain chemotherapeutic agents [32]. Mutations in proto-oncogenes or tumor suppressors, like Ras and p53, alters apoptosis signaling and changes the tumor microenvironment which traits of tumor cell resistance to therapy [33, 34] and subsequent tumor recurrence [35]. A large number of experimental studies have shown that apoptosis induced by irradiation is a determining factor of radiosensitivity [36].
5. *Molecular targeting agents* (*EGFR*, *COX2*, *and Mn-SOD*). Molecular targeting agents, such as EGFR, COX2, and Mn-SOD, may be possible to efficiently increase radiosensitivity of cancer cells when given with RT and also eradicate subclinical metastases by themselves [21].

The Role of Hedgehog Signaling in Cancer Treatment Resistance

HH ligands (Sonic, Desert, and Indian) bind to and antagonize the cell surface receptor PTCH, relieving the PTCH-mediated suppression of the transmembrane protein smoothened (Smo). Smo then initiates an intracellular signaling cascade that leads to the activation and nuclear translocation of the Gli family (Gli-1, 2, and 3). Gli family mediates transcription of genes controlling proliferation, differentiation, and survival of cells [1, 6]. Aberrations in hedgehog signaling have been found in cancers [37], resulting in overexpression of HH signaling pathway and an increase in endogenous production of HH ligands [4, 5]. Therefore, suppression of HH signaling might be a valid therapeutic option for overcoming drug resistance and for increasing the success of chemotherapy. Cui et al. analyzed 60 glioma samples, indicating that overexpression of Gli-1 is correlated with glioma recurrence after chemotherapy including VCR, VP16, CDDP, and ACNU [8]. Blockade of HH pathway enhanced cytotoxicity of chemotherapeutic agents in glioma cells through downregulating the expressions of MDR1, MRP1, MVP, MGMT, Bcl-2, and Survivin genes [38].

A growing body of evidence indicates that HH signaling plays an important role in regulating cancer treatment resistance. For example, IR-induced DSBs activate the PI3K-related kinases ATM and ATR, which regulate apoptosis, cell cycle progression, and DNA repair [39]. Research on basal cell nevus syndrome (BCNS; also known as Gorlin syndrome) patients and *Ptc1*$^{+/-}$ mice have shown a defect in the IR-induced activation of the ATR-Chk1 checkpoint signaling pathway (a pathway that serves as a barrier to the development of tumors), resulting in dramatically increases the incidence of tumors in *Ptcl*$^{+/-}$ mice [40]. Likewise, transient expression of Gli-1 disrupts Chk1 activation in human cells, suggesting that SHH signaling attenuates the activation of a genotoxin-triggered ATR–Chk1 checkpoint signal transduction pathway, and inappropriate SHH pathway activation promotes tumorigenesis by disabling a key signaling pathway that helps maintain genomic stability and inhibits tumorigenesis [40].

Induction of Tumor Regrowth and Cancer Stem Cells

Stem cells and CSCs share some features, including signaling pathways to regulate self-renewal and differentiation [41]. Similar to normal stem cells, CSC are thought to be relatively quiescent, to be resistant to drugs and toxins, and to possess the DNA repair capacity [42]. For radiation sensitivity in cancer cells, the vast majority of experimental and clinical studies support four determinant phenomena in radiobiology: repair of DNA damage, redistribution of cells in the cell cycle, repopulation, and reoxygenation of hypoxic tumor areas [43]. The effectiveness of each radiation fraction decreases with increasing repopulation of tumor cells, suggesting repopulation by an RT-resistant progeny [44]. The mechanisms that underlie accelerated repopulation are poorly understood, but may involve the proliferation of previously quiescent treatment-resistant clonogenic cells or, CSC [45].

Signaling pathways, such as the Bmi-1, Notch, Wnt, and SHH pathways [46, 47] that support the dysregulated self-renewal and proliferation of CSC may be targets for regulating tumor regrowth and improving treatment outcomes [48, 49]. The investigations of CSC signaling activation during tumor repopulation suggest that the SHH pathway is an important target to regulate proliferation of surviving clonogens after concurrent chemoradiotherapy (CCRT) [50]. A significant upregulation of SHH and Gli-1 expression was observed in the majority of residual tumors after chemoradiotherapy, suggesting that HH signaling may contribute to cancer resistance [4]. Treatment of cancer cell lines with the HH-inhibitory compound cyclopamine results in downregulation of the proliferation marker Ki67 and reduced proliferation rates [5, 50], indicating that HH pathway activation may be essential for tumor growth and maintenance. Smo knockout studies in chronic myeloid leukemia (CML) CSCs (Bcr-Abl-driven $Lin^{-}/Sca1^{+}/c\text{-}Kit^{+}$) cells reduced their ability to form tumors in irradiated mice [51]. Smo antagonists inhibit the growth of these CML CSCs in vitro and prolong survival in vivo, importantly also in cells with resistance to the currently used Bcr-Abl inhibitors imatinib or nilotinib, suggesting that combination therapy might help to prevent relapses in this chronic disease [51]. Sims-Mourtada et al. indicated that the SHH signaling pathway was extensively activated in both chemoradiotherapy-resistant residual esophageal carcinoma specimens and animal tumor xenografts, causing the promotion of tumor repopulation and contribute to chemoradiation resistance through upregulation of the G1-cyclin-Rb axis [4].

Anti-apoptosis and Cell Cycle Regulation

Research on SHH pathway has demonstrated that SHH contributes to the survival of cells by opposing the execution of intrinsic and extrinsic apoptotic cascades [52, 53]. Research in lymphocytes demonstrated that this effect of SHH signaling may go through the prevention of Fas-induced apoptosis [54]. Many malignant

cells remain resistant to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) induced apoptosis, and blockade of HH using pharmacological and genetic tools sensitized the cells to TRAIL cytotoxicity [55]. Small interfering RNA (siRNA)-targeted knockdown of Gli-3, but not Gli-1 or Gli-2, restored receptors death receptor 4 (DR4) expression and TRAIL sensitivity, suggesting that modulation of HH–Gli pathway might be a new therapeutic approach for TRAIL-resistant neoplasms [55]. In B-cell chronic lymphocytic leukemia (B-CLL), selective downregulation of Gli-1 by antisense oligodeoxynucleotides results in decreased Bcl-2 expression and increased apoptosis, suggesting that Gli-1 may regulate Bcl-2 and, thereby, modulate cell survival [56]. For tumor cells, inhibition of SHH signaling has been shown to induce apoptosis in tumors through the activation of both intrinsic and extrinsic apoptosis cascades [57], such as SHH signaling increased Bcl-2 expression in BCCs [58].

Additionally, the sensitivity of cells to the cytotoxic effects of radiation is cell cycle dependent, with the S-phase being more resistant and the G1-S boundary and G2/M phase being more sensitive [4]. Sims-Mourtada et al. demonstrated that the treatment of SEG-1 cells with ionizing radiation alone led to a slight but not significant reduction in the radiation-resistant S-phase fraction. Treatment with HH inhibitors alone led to a significant reduction in the S-phase fraction, and the combination of radiation and HH inhibitors caused a greater reduction in the S-phase fraction compared with untreated cells [4]. In a mouse xerograft model, SHH–Gli-1 signaling pathway was shown a high association with the increase in proliferation and repopulation of esophageal cancer observed after CCRT [4].

Repair of DNA Damage

DNA damage includes endogenous (such as oxidative metabolites) and external exposures (such as environmental pollution), causing single-strand breaks (SSB) and DSBs that may limit survival and the regenerative potential of cells [29]. Repair of DNA DSB can occur via nonhomologous end joining (NHEJ) or homologous recombination (HR). HR is required for a sister chromatid present in the S/G2 phase of replicating cells to provide an error-free template for DNA repair [59] while NHEJ is an error-prone repair mechanism that enzymatically modifies the two ends of a DNA break so that they are compatible for direct ligation [60]. UV light is known to induce DNA repair in irradiated cells through the upregulation of damaged DNA-binding (DDB) proteins, DDB1 (127 kDa) and DDB2 (48 kDa), which mediate a key process in nucleotide excision repair after UV damage [61, 62]. EGFR is also involved in DNA synthesis and DNA repair through its interactions with DNA proliferating cell nuclear antigen (PCNA) [63] and DNA-dependent protein kinase (DNA-PK), which is required for DNA repair [64]. Other factors, such as ionizing radiation, heat, H_2O_2, and cisplatin treatment, induce Ku70/80 and phosphatase I translocation to the nucleus and increase DNA-PK activity for initiation of DNA repair [64–66].

Drug resistance can, in some cases, be attributed to increased DNA repair response but may also result from a variety of other alterations, including decreased apoptotic signaling in response to this form of DNA damage [67, 68]. Articles reported that p53- and DNA mismatch repair (MMR) deficiency are two key genetic changes that have been associated with resistance to cisplatin [68, 69]. Frappart et al. have demonstrated that inactivation of the DNA repair factors, together with p53 loss, led to rapid medulloblastoma formation [60]. Genomic analysis of the tumors showed recurring chromosome 13 alterations via chromosomal loss or translocations involving regions containing *Ptch1*. Sequence analysis of the remaining *Ptch1* allele showed a variety of inactivating mutations in all tumors analyzed, highlighting the critical tumor suppressor function of this hedgehog-signaling regulator and Ptch1 tumor suppressor activity [60]. Moreover, mutations of multiple genes involved in the SHH pathway (including *PTCH1*, *SUFU*, *Smo*) or the Wingless (*WNT*) pathway (such as *AXIN1* or β-*CATENIN*) have also been found in sporadic human medulloblastomas, the most common malignant pediatric brain tumor, highlighting the importance of these pathways for preventing cancer [70]. Couvé-Privat et al. (2002) demonstrated that the presence of relatively high levels of ultraviolet-specific mutations in the *Smo* proto-oncogene in BCC from DNA repair-deficient xeroderma pigmentosum patients has confirmed its importance in BCC development [71]. Research on engineered loss of Pten or expression of a constitutively active Akt can synergize with engineered dysregulation of SHH signaling in mouse models to generate medulloblastoma [72]. Both pathways were targeted by somatic changes arising in medulloblastoma with defective HR, which showed abnormalities in Pten and PI3K signaling in combination with biallelic inactivation of *Ptch1* [60]. Shafaee et al. reported that cyclopamine increased the cytotoxic effects of paclitaxel and ionizing radiation in HH expressing pancreatic carcinoma cells [73]. Although potential interactions between DNA repair mechanisms and the HH pathway is suspected, the radiosensitizing mechanism of cyclopamine is still not fully understood [74].

Stimulation of Multiple Drug Resistant Transporter System

Multidrug resistance (MDR) is a common problem in cancer chemotherapy, resulting from enhanced drug efflux from cancer cells mediated by members of the ATP-binding cassette (ABC) transporter family [75]. Permeability-glycoprotein (P-gp), a product of the multidrug resistance gene 1 (*mdr1*), is one of the best characterized MDR molecules, which highly expressed in solid tumors and, moreover, in CSCs [75]. Recently, research demonstrated that imatinib mesylat (IM), a specific tyrosine kinase inhibitor commonly used in CML, was a substrate of P-gp so that *mdr1* gene overexpression can confer resistance to it [76, 77].

Constitutive activation of the HH pathway has been shown to contribute to the growth and maintenance of various cancers [78]. Previous studies have

shown that the HH pathway regulates cell cycle progression and apoptotic resistance; this likely contributes to HH-induced chemoresistance [4, 5]. Sims-Mourtada et al. show that HH signaling regulates the expression of the ABC transporter protein P-gp and breast cancer resistance protein (BCRP), and blockade of SHH activation by cyclopamine or a Gli-1 specific siRNA resulted in decreased expression of these transporters [74, 78]. In addition, simultaneous treatment of SHH ligand and cyclosporine A, a broad inhibitor of ABC transporter function, blocked this decrease of drug uptake in SEG-1 esophageal adenocarcinoma cells for [3] H-labeled Taxol, MTX, and VP-16 [78]. These findings suggest that SHH signaling may promote MDR via increasing drug efflux by ABC transporters [75].

Development of HH Regulating Therapeutics

It has been shown that topical application of cyclopamine inhibited the growth of human BCC [79]; however, concerns of neurological disturbances may limit the systemic application of this drug. Cyclopamine and other compounds, such as cyclopamine derivatives IPI-926 and Cyc-T, and synthetic compounds GDC-0449, XL-139, LDE-225, SANT1, and Cur-61414, action in binding to and antagonizing Smo. SHH-neutralizing antibodies and Robotnikinin were reported that block the SHH pathway by directly targeting SHH, while arsenic, HPI-1, HPI-2, GANT-56, and GANT-61 were potent Gli inhibitors [80–82]. Several small molecule compounds that prevent HH signaling by binding to and inhibiting Smo are currently under development, including Cur-61414 [83], which has shown promising results in the inhibition of BCC and pancreatic cancer in preclinical models. Vismodegib (GDC-0449, discovered by Genentech Inc. under collaboration with Curis Inc.) [84] is a small, orally administrable molecule with suppression effect on HH signaling by binding to and interfering with Smo. Preclinical studies of vismodegib in mouse models of medulloblastoma and in xenograft models of colorectal and pancreatic cancer, and phase I clinical trials in patients with advanced BCC and MB highlighted an objective response to vismodegib [84, 85]. Because of its low toxicity (with only one grade 4 adverse side effects) and specificity for the HH pathway, vismodegib is currently undergoing phase II clinical trials for the treatment of more solid tumors, and may also be used in combination treatments with conventional chemotherapy [84]. Although systemic inhibitors of HH signaling have been undergoing clinical trials, the discovery may provide potentially a novel therapeutic strategy in tumors because HH signaling blockade may not only impair tumor proliferation, but may increase chemotherapeutic efficacy, and result in improved treatment responses. The therapeutic effects of HH pathway blockade in combination with current CRT regimens are perspective to be investigated in the future. The differential regulation and timing of HH activity in normal and tumor tissue after CRT should also be investigated to optimize the most beneficial therapeutic index.

Concluding Remark

HH signaling increases the resistance of cancer cells to radiotherapy, chemotherapy, and CRT. Research results demonstrate that HH signaling confer treatment resistance of cancer cells through four aspects, including the induction of tumor regrowth and CSCs, anti-apoptosis and cell cycle regulation, modulation of DNA damage repair, and stimulation of MDR transporter system. Inhibition of HH activity may sensitize tumor cells to radiation and chemotherapeutic drugs to improve the treatment outcome [4].

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