Cancer therapy that targets the Hedgehog signaling pathway considering the cancer microenvironment (Review)

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Abstract. Recently, the cancer microenvironment (CME) has received significant attention. At the local site of the tumor, cancer progression is affected by secreted cytokines and conditions derived from the CME and stimulation by cancer-induced cytokines in an autocrine manner. The CME is characterized by various types of conditions, such as hypoxia, inflammation stimulation, and angiogenesis, and contains various components, such as reactive oxygen species, cancer-associated fibroblasts, infiltrated immune cells, exosomes, and cancer stem cells (CSCs). These conditions and components complicate the progression of cancer. The Hedgehog (HH) signaling pathway is a morphogenesis signaling pathway that is reactivated in some cancers. In these cancers, reactivated HH signaling is involved in the induction of the malignant phenotype. HH signaling is also activated under hypoxic conditions and is considered to be strongly correlated with the CME, including the induction of cancer fibrosis and maintenance of CSCs. The aim of the present review was to elucidate a cancer therapy that targets HH signaling by considering the CME, particularly focusing on hypoxia.

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1. How is the cancer microenvironment and Hedgehog signaling important for the cancer therapy?

The CME is an extremely special environment that favors cancer progression. The CME is characterized by various conditions, such as hypoxia and angiogenesis, and contains several components, such as cancer-associated fibroblasts (CAFs), infiltrated immune cells, and cancer stem cells (CSCs) (1-3). These conditions and components affect each other, establishing a cancer-specific environment. The actual conditions of the CME are complex and multifactorial. Hypoxia is considered to be an important factor in the CME. Hypoxia regulates various factors and conditions of the CME. The signaling pathways and molecules that are not activated under normoxia may be activated under hypoxia. Cancer may induce a malignant phenotype through hypoxia-activated signaling pathways, such as the Hedgehog (HH) signaling pathway, and molecules. HH signaling is involved in the CME and is activated under hypoxic conditions (4). Moreover, the contribution of HH signaling to cancer shifts from gene mutation to a ligand-dependent paracrine manner via surrounding conditions such as the CME (5). Therefore, the importance of the CME in HH signaling activation has received significant attention. Treating cancers using only a single therapeutic method is considered difficult. Various processes in cancer progression that are observed in the CME may be involved in this refractory mechanism. It is considered that an in-depth understanding of the various conditions observed in the CME and measures to prevent these conditions will contribute to the development of new effective cancer therapies for the next generation. To understand the CME, the individual factors that constitute the CME should be first elucidated.

2. What is the Hedgehog signaling pathway?

The HH signaling pathway is a morphogenesis signaling pathway that plays a pivotal role in growth and pattern during the embryonic period (6). However, it may be reactivated

beyond the embryonic period in certain cancers, which acquire a malignant phenotype via HH signaling. Core components of HH signaling that are emphasized in the present review are the 12-transmembrane and negative regulatory receptors, Patch (PTC), 7-transmembrane protein and Smoothened (SMO), 3 Hh ligands including sonic HH (SHH), Indian HH (IHH) and desert HH (DHH), serine-threonine kinase, FUSED, suppressor of FUSED (SUFU), and the 3 transcriptional factors, glioma-associated oncogene (GLI)1, GLI2 and GLI3. In the absence of HH ligands, PTC inhibits SMO and GLIs form huge complexes with FUSED and SUFU. Therefore, GLIs cannot translocate to the nucleus, and the signal does not transduce. In contrast, in the presence of HH ligands, SMO is released from the inhibition of PTC, and then, GLIs can be released from the complex. Thereafter, GLIs can translocate to the nucleus, and signaling is successfully transduced. Target genes of HH signaling include GLI1 and PTC1. Therefore, GLI1 is considered to be an index of HH signaling activation (7). Fig. 1 shows an outline of HH signal transduction. The mechanism of reactivation of HH signaling in cancers is considered to be gene mutation. For example, there are certain reports of gene mutations in HH signaling in basal cell carcinoma (8), medulloblastoma (9), rhabdomyosarcoma (10) and glioblastoma (11). After 2003, ligand-dependent HH signaling activation, but not gene mutation, has been reported. For example, SHH secreted in an autocrine or paracrine manner from the CME activates HH signaling in pancreatic cancer (12), colon cancer (13), hepatocellular carcinoma (HCC) (14), lung (15), ovarian (16), gastric (17) and prostate cancer (18). Previously, it was revealed that SHH, from monocytes that exist in pancreatic cancer stroma, activates HH signaling in pancreatic cancer to induce proliferation (19). HH signaling activation by SHH secreted from the adjacent tissue is a more severe problem than gene mutation from the viewpoint of the high probability of induction of HH signaling activation. This may also be a reason why determining the association between the HH signaling pathway and the CME is important.

3. Hypoxia

Molecules and signaling pathways that are activated under hypoxia. Cancer hypoxia is an important characteristic of the CME. Hypoxia is ordinally investigated under 20% O_2 conditions, however, 20% O_2 conditions do not exist *in vivo*. The O_2 saturation of all human tissues is ~1% O_2, and cancer tissue is particularly hypoxic (O_2 saturation, ~0.1%) (20). The molecules and signaling pathways that are not activated under normoxic conditions may be activated under hypoxic conditions. To determine the cancer phenotype under hypoxic cancer conditions, 1% O_2 is used in experiments. It has been previously reported that activation of HH signaling is upregulated under hypoxic conditions (4). In the present study, SMO transcription increased under hypoxic conditions. A similar result was reported by Lei *et al* (21). In the analysis of the mechanism underlying the increase in SMO expression under hypoxia, the upstream molecules of SMO were analyzed and two molecules, recombination signal binding protein for immunoglobulin-kappa-J region (RBPJ) and mastermind-like 3 (MAML3), were detected (22). RBPJ and MAML3 have recently been found to be a transcriptional factor and a coactivator of Notch signaling, respectively (23). The Notch signaling pathway is also a morphogenetic signaling pathway. Our previous study on pancreatic cancer cell lines revealed that hypoxia increases the expression of RBPJ and MAML3 and contributes to the transcription of SMO (22). This RBPJ/MAML3/SMO signaling pathway is also activated in small-cell lung cancer (24). The RBPJ/MAML3/SMO signaling may be a comprehensive therapeutic target for morphogenesis signaling. Hypoxia-inducible factor (HIF)-1a is an important transcriptional factor that plays a pivotal role in various cell functions such as cell proliferation, survival, apoptosis, and angiogenesis under hypoxia. No correlation or crosstalk was observed in RBPJ/MAML3/SMO signaling in our previous study (22). However, numerous studies have shown a correlation between HIF-1α and HH signaling. Considering that HIF-1α regulates HH signaling as an upstream mediator, it was demonstrated that fibroblast growth factor receptor-like-1 (FGFRL1) expression is induced by HIF-1α and that it promotes tumor progression by crosstalk with HH signaling in ovarian cancer (25). Mitochondrial glutamic pyruvate transaminase was revealed to promote tumorigenesis and stemness of breast cancer cells by activating HH signaling via HIF-1α (26). In addition, it has been reported that natural agents contribute to the interaction between HIF-1α and HH signaling. Resveratrol, which is extracted from various plants, decreased HIF-1α expression and inhibited HH signaling to decrease invasiveness in gastric cancer cells (27). Oroxylin A, a bioactive flavonoid, induced HIF-1α degradation and led to the inactivation of HH signaling to increase the sensitivity of glioma cells to temozolomide (28). Curcumin has an inhibitory effect on HIF-1α, decreasing proliferation in breast cancer (29), and curcumin was revealed to suppress hypoxia-induced endothelial-mesenchymal transition (EMT) by inhibiting HH signaling in pancreatic cancer cells (30). HIF-1a protects cancer cells from radiation-induced apoptosis (31). Furthermore, curcumin has been shown to increase the efficiency of g-irradiation in glioma by suppressing HH signaling (32). This suggests that the curcumin-induced decrease of HIF-1α may lead to inactivation of HH signaling and consequently suppression of cancer cell function. Conversely, HH signaling has been reported to regulate HIF-1α. In a previous study, inhibition of HH signaling suppressed hepatic stellate cells through inhibition of HIF-1α and heat shock protein 90 (33).

Correlation of hypoxia with other morphogenesis signaling pathways. Other morphogenesis signaling and HH signaling pathways have been associated to the CME. The correlation between hypoxia and Wnt/b-catenin signaling has been well elucidated. Among the three subunits of HIF (HIF-1α, 2α and 3α), the contribution of HIF-2α in tumor progression is well reported in Wnt/β-catenin signaling (34). Inhibition of HIF-2α leads to decreased expression of β-catenin and SMAD4 and suppresses the progression to high-grade mPanINs (35). However, the precise contribution of hypoxia to Notch signaling activation has not been clearly reported. As previously described, hypoxia induces the expression of RBPJ and MAML3 (22). Considering that RBPJ is a transcriptional factor for Notch signaling and MAML3 is a transcriptional mediator of Notch signaling, Notch signaling should be activated under hypoxia. Moreover, RBPJ and

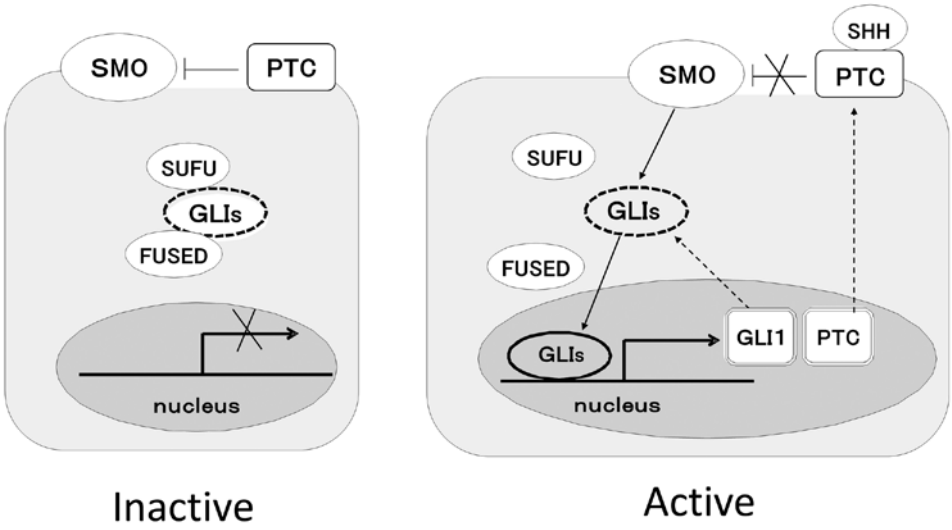

Figure 1. Schematic representation of signal transduction in HH signaling. In the absence of SHH, PTC inhibits Smoothened and GLIs form a huge complex with FUSED and SUFU. Therefore, GLIs cannot translocate to the nucleus and the signal cannot be transduced (left panel). However, in the presence of SHH, SMO is released due to the inhibition of PTC, and GLIs can be released from the complex. GLIs can translocate to the nucleus to successfully transduce the signal (right panel). HH, Hedgehog; SHH, sonic HH; PTC, Patch; SMO, Smoothened; GLIs, glioma-associated oncogenes; SUFU, suppressor of FUSED.

MAML3 may regulate HH signaling and Notch signaling concurrently. RBPJ and MAML3 could be new comprehensive therapeutic targets for morphogenesis signaling. In another study, activated Notch1 markedly increased the transcriptional activity of HIF-1 (36). In choriocarcinoma cells, it has been shown that HIF-1α promotes invasiveness through Notch signaling activation (37). These results explain the association between hypoxia and Notch signaling. FGF signaling is also a type of morphogenesis signaling. Although a direct correlation between hypoxia and FGF signaling has yet to be demonstrated, hypoxia is involved in the activation of different signaling pathways in FGF-2-stimulated human microvascular endothelial cells, which may contribute to hypoxia-induced angiogenesis (38). FGF signaling is also a key pathway in HCC (39). Therefore, FGF signaling plays an important role in the progression of cancer in the CME.

Acidosis and reactive oxygen species (ROS). Acidosis and ROS are among the most characterized properties of the CME. Hypoxic conditions are considered to induce ROS generation and cause acidosis. Acidosis also induces ROS generation (40). ROS contribute to transformation, survival, proliferation, invasion and metastasis of cancer cells (41). Although the correlation between acidosis and HH signaling has not been fully elucidated, it has been shown that ROS promotes HIF-1α stabilization to induce HH signaling activated-cancer cell proliferation (42). Other studies have revealed that ROS inhibitors block GLI1-dependent EMT and invasion under hypoxia (43) and that resveratrol suppresses hypoxia-induced ROS-mediated invasiveness and migration in pancreatic cancer via inhibition of HH signaling (44).

Reoxygenation. Reoxygenation is an important process in the CME. It is considered to be the process by which cancer cells detach from hypoxic cancers and metastasize to secondary tissues through the bloodstream. HH signaling may contribute to cancer progression during reoxygenation. In a previous study, pancreatic cancer cells increased proliferation and invasion during the reoxygenation process through HH signaling activation using the chronic hypoxia-resistant pancreatic cancer cells that were generated (45). Consistent with this result, it has been reported that the activation of HH signaling protects cell apoptosis and cell viability from reoxygenation stress in noncancerous H9C2 myocardial cells and HK-2 cells in experiments assuming the clinical situation of ischemia (46,47). Therefore, reoxygenation-induced HH signaling activation may be required for tissue repair. Cancer may utilize this nature of HH signaling during the reoxygenation process.

Angiogenesis. Cancer adapts to hypoxic conditions by inducing the formation of new blood vessels, which is called angiogenesis. Angiogenesis is implicated in hypoxia. Angiogenesis-related genes include vascular endothelial growth factor (VEGF), VEGF receptor, basic FGF (bFGF), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), adrenomedullin, and epidermal growth factor (EGF); these genes are targets of HIF (48).

HH signaling contributes to vasculature development, differentiation, and maintenance during the embryonic period (49). Canonical HH signaling has been reported to regulate hepatic stellate cell-induced angiogenesis in liver fibrosis (28). Yang *et al* (50) have revealed that HH signaling, prospero-related homeobox 1, and HIF-1α contribute to liver sinusoidal endothelial cell angiogenesis. Considering this fact, HH signaling appears to affect vasculature development even in cancer tissues. The association between HH signaling and VEGF has been reported in several types of cancers, such as HCC (51) and colorectal cancer (52). The association between HH signaling and bFGF has been reported (53), and it may also be related to cancer fibrosis induced by HH signaling, as described below. The association between HH signaling and PDGF (54), IGF (55), and EGF (56) has also been reported. Bausch *et al* (57) have shown that SHH stimulates angiogenesis indirectly through other pathways,

including the reduction of antiangiogenic thrombospondin 2 and tissue inhibitor of metalloproteinase 2 in stromal cells in pancreatic cancer. Thus, angiogenesis, hypoxia, and HH signaling are well correlated.

4. Cancer fibrosis

Cancer fibrosis is an important process and a complication in which cancer acquires the refractory phenotype. The association between HH signaling and fibrosis has been implicated in chronic lung fibrosis in 2003 (58) and biliary fibrosis in chronic cholecystitis in 2008 (59). Fibrosis is marked in pancreatic cancer, and desmoplasia has been investigated. HH signaling has been reported to promote desmoplasia in pancreatic cancer in 2008 (60). Recently, it was demonstrated that the increased secretion of SHH through HIF-1α signaling is responsible for the cancer fibrosis or the stroma-rich environment in pancreatic cancer (61,62). A severe case of cancer fibrosis in the CME may block the circulation of chemotherapeutic agents and infiltration of immune cells. Olive *et al* (63) have revealed that inhibition of HH signaling enhances the delivery of chemotherapy in a pancreatic cancer mouse model. In our xenograft experiments using pancreatic cancer cell lines and CAFs, inhibition of cancer fibrosis by HH inhibition led to an increase in tissue-infiltrating lymphocytes and an enhancement of the effect of immune checkpoint inhibitors (64). However, Steele *et al* (65) have shown that inhibition of HH signaling reduces myofibroblastic CAFs and increases inflammatory CAFs to decrease cytotoxic T-cell infiltration and expand regulatory T (Treg) cells. There are few studies on infiltration of dendritic cells (DCs) and macrophages related to cancer fibrosis. However, some researchers have shown that macrophage infiltration induces fibrosis. Xue *et al* (66) revealed that macrophages promote pancreatic fibrosis in chronic pancreatitis, and Ueshima *et al* (67) demonstrated that macrophage-secreted transforming growth factor (TGF)-1 contributes to fibroblast activation. Cancer fibrosis consists of CAFs and an extracellular matrix that secretes various cytokines. One of the most important cytokines is TGF-β. Fibrosis is a typical pathological condition of TGF-β-driven disease (68). The TGF-β/SMAD cascade is considered to be a potent inducer of GLI2 (69). Therefore, in the presence of TGF-β, it may induce cancer fibrosis and activate HH signaling, which may lead to more fibrosis. Zhou *et al* (70) showed that HH signaling and TGF-β1 contribute to the progression of fibrosis in nonalcoholic steatohepatitis. A previous study revealed the association among TGF-β, fibrosis, and HH signaling, particularly in liver fibrosis, and GANT61, a GLI inhibitor, has been shown to be effective for liver fibrosis (71). Both HH signaling and TGF-β in the CME may play an important role in cancer fibrosis.

5. Immune cells

As aforementioned, the CME is closely correlated with hypoxia and HH signaling activation. Therefore, the functions of immune cells such as lymphocytes, macrophages, DCs, myeloid-derived suppressor cells (MDSCs), and Treg cells that infiltrate local cancer sites should be considered with regard to these factors.

Association to hypoxia. It has been shown that hypoxic stress increases the cytotoxicity of $CD8^+$ T cells and decreases their proliferative and differentiating capacities (72). Consistent with this result, in our previous study, proliferation of activated lymphocytes decreased under hypoxia, but there was no significant change in their migration (73). The function of DCs is also altered under hypoxia. The duration of hypoxic exposure may affect the DC response and continuous vs. intermittent hypoxia (74). The motility and phagocytic ability of hypoxia-induced DCs are significantly lower than those of normoxia-induced DCs. Maturation of hypoxia-induced immature DCs is more suppressed than that of normoxia-induced immature DCs due to their decreased motility and phagocytosis (75). In addition, previous studies have shown that the cross-linking of triggering receptors expressed on myeloid cells-1 under hypoxia is associated with an induced release of inflammatory cytokines and chemokines in DCs (76,77). With respect to immune-suppressing cells, it has been reported that hypoxia induces CCL28 to recruit Treg cells at the local site of the cancer (78). It has also been revealed that hypoxia enhances immunosuppression by promoting immunosuppressive capacities of Treg cells (79). Similarly, hypoxia induces MDSC recruitment through CCL26 in HCC (80). It has been identified that HIF-1α regulates the function and differentiation of MDSCs within the hypoxic CME (72). HIF-1α plays a pivotal role in macrophage-mediated inhibition of T cells under hypoxia (81). Macrophages also upregulate the expression of matrix metalloproteinase-7 in hypoxic tumor cells to protect tumor cells from the cytotoxic activity of natural killer cells and T cells (82,83). In a study by Sureshbabu *et al* (84), hypoxia-exposed γδT cells exhibited reduced cytotoxicity in oral tumor cells. Thus, hypoxia mainly supports the immunosuppressive function of immune cells.

Association to HH signaling. HH signaling contributes to the function of activated lymphocytes, such as migration, proliferation and cytotoxicity (73). T-cell receptor activation triggers the expression of HH signaling components, and HH signaling is required for cytotoxic T lymphocyte (CTL) killing (85). Conversely, certain researchers have shown that HH signaling promotes tumor-associated macrophage polarization to inhibit tumor-infiltrated CD8 T-cell recruitment (86) and that HH signaling promotes Th2 differentiation in naive human CD4 T cells (87). Other researchers have shown that GLI1 induces the polarization of invading myeloid cells to MDSCs (88). These results indicated that HH signaling is required for lymphocyte function and immune response in both activation and inhibition. HH signaling is also involved in the functions of DCs, including induction, migration, chemotaxis, phagocytosis, maturation, and IL-12 p40 or p70 secretion and the allogeneic lymphocyte stimulation activity of monocyte-derived DCs (89). The association between hypoxia and HH signaling may determine the functions of immune cells.

Correlation between the programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) axis and the CME. Previously, the concept of immune checkpoints has received significant attention. There are patients who are not eligible to receive standard therapy due to their drug tolerance and achieve complete response by immune checkpoint inhibitor

treatment (90). Thus, studies on the PD-1/PD-L1 axis are considered important. It has been shown that PD-L1 is a direct target of HIF-1α (91). Hypoxia-induced PD-L1/PD-1 crosstalk impairs T-cell function (92). Tumors may escape immune cells by regulating the PD-1/PD-L1 axis under hypoxic conditions. However, it has been shown that HH signaling induces PD-L1 expression in gastric cancer (93) and that HH inhibition leads to a decrease in PD-L1 expression under hypoxia in pancreatic cancer (94). Previously, it has been shown that soluble PD-1/PD-L1 or exosomal PD-L1 plays an important regulatory role in antitumor immunity (95,96). Development of a measure against the enhanced PD-1/PD-L1 axis should be the next strategy for cancer therapy. With respect to the other factors of the CME, lymphocytes secrete INF-γ, which induces PD-L1 when lymphocytes infiltrate the cancer tissue (97). A previous study has shown that PD-L1 expression is associated with tumor-infiltrating lymphocytes in squamous cell cervical carcinoma (98). However, it is unclear whether lymphocyte infiltration into cancer tissue is the cause or result of PD-L1 expression. In addition, although PD-L1 is considered to be an exhaustion marker (99), the significance of PD-L1 expression as a biomarker for immunotherapy is controversial.

6. Autophagy

Autophagy is a cellular self-degradation process that maintains homeostasis using this system. Autophagy is involved in the initiation, progression, and drug resistance of cancers (100); therefore, autophagy is considered a target for cancer therapy. Hypoxia and metabolic stress upregulate autophagy (101). Autophagy and hypoxia-upregulated HH signaling appear to be correlated, and the association between autophagy and HH signaling has been well elucidated (102,103). However, it is unclear whether HH signaling inhibits or upregulates cancer autophagy. The SMO antagonist vismodegib was demonstrated to trigger marked autophagy in non-small cell lung cancer (104), while the GLI1/2 inhibitor GANT61 induced autophagy in HCC (105). Milla *et al* (102) have shown that the HH antagonist cyclopamine prevents autophagy. Further, Gagné-Sansfaçon *et al* (106) have revealed that loss of HH signaling leads to a decrease in autophagy in the intestinal ileum. Therefore, it is deemed that the contribution of HH signaling to autophagy warrants further investigation, considering the fact that there is crosstalk between HH signaling and other signaling pathways.

7. Cancer stem cells

Increasing evidence suggests that the host microenvironment plays a pivotal role in CSC status (107). For example, hypoxia promotes stem-like properties of laryngeal cancer cells (108) and is closely associated with the resistance of CSCs to chemotherapy and radiotherapy (109). Hypoxia enhances the expression of the CSC transcription factors NANOG, Oct4, SOX2 and CD133 (110). Multiple secreted cytokines and growth factors in the CME induce the enrichment of CSCs in ovarian cancer (111). As with other CME factors, nutritional stress in the microenvironment induces increased expression of glioblastoma CSC-specific biomarkers with higher invasiveness and angiogenesis through Wnt/HH signaling (112). In addition, numerous studies have shown that morphogenesis signaling is important for the maintenance of CSCs. For example, in an experiment on breast cancer, the HH signaling pathway was activated in the $CD24^{-/low}$ $CD44^{+}$ CSC population, but not in the $CD24^{+}$ $CD44^{+}$ non-CSC population, and HH signaling inhibition in the $CD24^{-/low}$ $CD44^{+}$ CSC population attenuated tumor proliferation (113). Notch signaling contributes to endocrine resistance in breast cancer through the promotion of the CSC phenotype (114). Notch inhibitors increase the chemotherapy effect through $CD133^{+}$ CSC inhibition in endometrial cancer (115). Inhibition of Wnt/β-catenin signaling is considered to decrease the aggressiveness of breast cancer through CSC inhibition (116). Wnt/β-catenin signaling contributes to CSC-initiated HCC (117). Bone morphogenetic protein (BMP)/TGF-β signaling, which is a morphogenesis signaling pathway, contributes to the homeostasis of neural and glioma stem cells (118). The correlation between Notch signaling and BMP/TGF-β signaling has also been reported (119).

8. Exosomes

Exosomes are extracellular microvesicles measuring 30-100 nm in diameter, are actively secreted through an exocytosis pathway by various cell types (120,121), and comprise a nucleic acid and protein derived from secreted cells. Exosomes are significantly rigid and resistant to enzymatic degradation; therefore, they are considered to play a pivotal role in cell-to-cell interactions in the CME. Deep and Panigrahi (122) have reported that exosomes mediate tumor microenvironment remodeling, such as angiogenesis, EMT, metastasis, survival, proliferation, metabolism, stemness, and therapeutic resistance under hypoxic conditions through several signaling pathways, including the HH signaling pathway. Even during the morphogenesis period, exosomes are required for the distribution of morphogenes, such as HH ligands (123). In relation to HH signaling and CSC, it has been shown that exosomes derived from human bone marrow mesenchymal stem cells promote the growth of osteosarcoma and gastric cancer through HH signaling (124). CSC-derived exosomes contain stemness-specific proteins, self-renewal-promoting miRNAs, and survival factors, and they play a significant role in tumor heterogeneity and tumor progression (125). HH pathway proteins, including PTC1, SMO, and SHH, are exported to the cervical cancer cell line (126). SHH is highly expressed in CAFs, and CAF-derived exosomes contribute to the augmentation of growth and progression in esophageal squamous cell carcinoma (127). With respect to the association between exosomes and CME, Wada *et al* (128) have shown that TGF-β1 expressed on the surface of cancer ascites-derived exosomes is involved in the maintenance of the number and suppressive function of Treg cells. Matsumoto *et al* (129) have shown that dendritic cell-derived exosome supports CD4+ T cell survival. Taken together, exosomes play a pivotal role in the maintenance of CME.

9. Nuclear transcription factor-κB

Local cancer sites often arise from inflammation. Inflammation is closely related to the CME and is required for the initiation of immune cell activation. Nuclear transcription factor (NF)-κB is an important transcriptional factor that

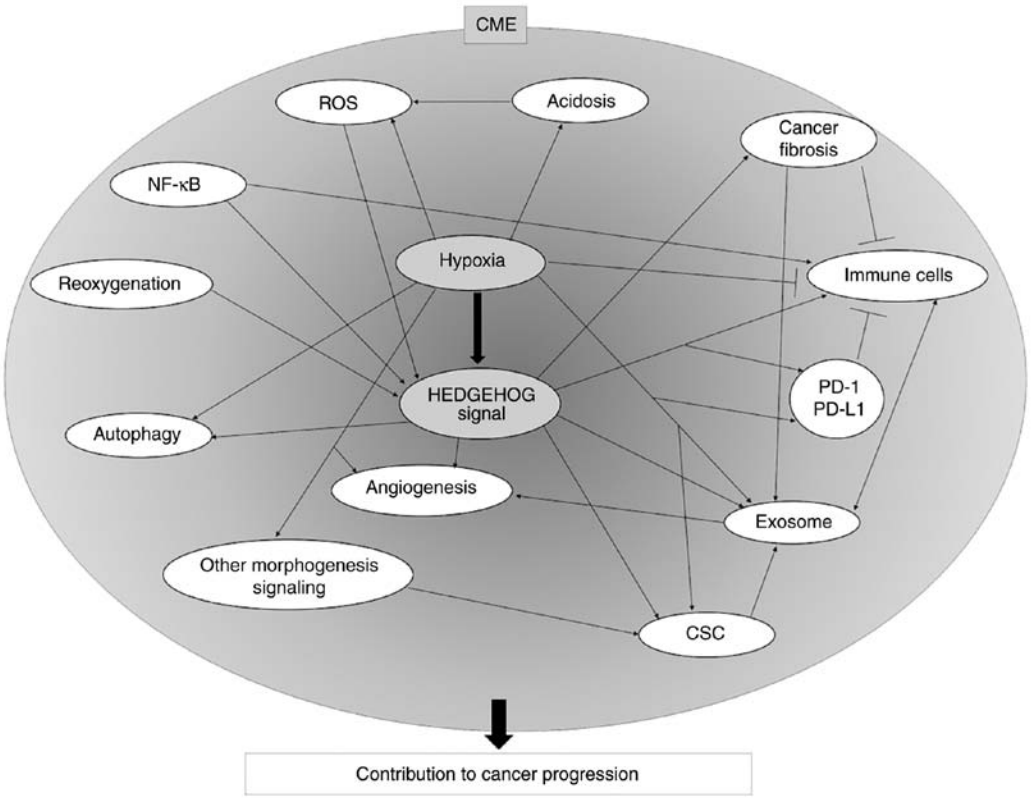

Figure 2. Schematic representation of the correlation of various CME factors in the CME. The present review focused on hypoxia and Hedgehog signaling. Each factor individually plays a pivotal role in the formation of the CME. The individual factors are correlated, form the CME and are involved with the cancer progression. CME, cancer microenvironment; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; ROS, reactive oxygen species; NF-κB, nuclear transcription factor-κB; CSC, cancer stem cell.

regulates inflammation (130). The association between SHH and NF-κB has been mainly reported. In a previous study, NF-kB was shown to contribute to the initiation of chronic pancreatitis and be involved in cancer initiation through SHH expression in pancreatic cancer (131). A similar finding was reported by Kasperczyk *et al* (132). The correlation between SHH and NF-κB has been revealed in multiple myeloma (133). SHH is secreted by tumor-infiltrated macrophages through the NF-κB pathway and induces proliferation in a paracrine manner in pancreatic cancer (19). The contribution of NF-κB to cancer-infiltrated lymphocytes has also been reported (134). Collectively, NF-κB significantly contributes to the CME.

10. Future directions

In the present review, the individual factors that constitute the CME have been described, focusing on hypoxia and HH signaling. As previously described, these factors are correlated and form the CME (Fig. 2). Inhibitors of each factor have been developed, and the mechanisms involved should be understood considering the complex correlation among the factors. Fosko *et al* (135) have revealed that vismodegib exhibited 60% response in basal cell carcinoma regardless of the histopathologic subtype. On the other hand, a phase 2 trial using the SMO inhibitor vismodegib with gemcitabine and nab-paclitaxel in patients with untreated metastatic pancreatic adenocarcinoma did not show a significant effective result (136). This trial had difficulties in analyzing the specimens before and after chemotherapy; the cause of the failure was not clear. Previous studies have shown that SMO mutation in cancer cells affects the effects of vismodegib (137,138). Thus, although HH inhibitors have exhibited a significant tumor suppressive effect *in vitro* (139), this effect has not always been observed *in vivo*. It was hypothesized that this discrepancy may be due to the difficulty in obtaining similar results with human CME as in *in vitro* and *in vivo* mouse experiments.

The therapy that targets only one CME factor may not be sufficient for cancer treatment. If the correlation among these CME factors can be substantiated, each CME inhibitor can be used effectively for cancer therapy. In Fig. 2, an overview of the correlation among hypoxia, HH signaling and other CME factors is demonstrated. Each factor individually plays a pivotal role in the formation of the CME. The correlated factors constitute the CME and contribute to cancer progression.

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Availability of data and materials

The datasets used and/or analyzed are available from the corresponding author on reasonable request.

Authors' contributions

HO, KatN and KY wrote the manuscript. SN, KazN and YO designed the manuscript. AF, KO and AY selected the references. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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