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Cancer Metastasis Facilitated by Developmental Pathways: Sonic Hedgehog, Notch, and Bone Morphogenic Proteins

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Abstract This review will highlight the significance of three critical pathways in developmental biology and our emerging understanding of their roles in regulating tumor metastasis: Bone morphogenic protein (BMP), Notch and Sonic hedgehog (SHH). We will discuss parallels between their known roles in development and how these processes can be used by tumor cells to create microenvironments that enhance tumor metastasis. That tumor cells usurp pathways critical to the developing embryo is not surprising, as many of the normal developmental programs include processes that are also seen during tumor progression to a metastatic phenotype, including epithelial to mesenchymal transition (EMT), tissue specific morphogenesis, cellular motility and invasion. BMPs are involved in EMT, contribute to tissue specific morphogenesis, and are expressed in highly-metastatic tumor cells. BMPs have also been hypothesized to have a role in the establishment of a pre-neoplastic niche. Notch and SHH facilitate neovascularization, angiogenesis, EMT and can contribute to the maintenance of highly-metastatic tumor stem cells. J. Cell. Biochem. 102: 829–839, 2007.
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Key words: metastasis; Sonic hedgehog; bone morhogenic protein; Notch

Tumor metastasis is responsible for 90% of deaths of cancer patients, yet remains one of the most poorly understood aspects of the pathogenesis and progression of cancer [Weigelt et al., 2005]. The aggressive tumor cells that metastasize from the primary tumor often usurp pathways that function during normal development. Embryonic pathways are believed to affect the survival of tumor stem cells and to orchestrate a complex microenvironment that promotes tumor survival and progression. Understanding these pathways will provide critical insight into the mechanisms of tumor metastasis, which heralds great promise for the discovery of novel therapeutics and the treatment of metastatic disease.

This review will highlight the significance of three critical pathways in developmental biology and our emerging understanding of their roles in regulating tumor metastasis: Bone morphogenic protein (BMP), Notch and Sonic hedgehog (SHH). We will discuss parallels between their known roles in development and how these processes can be used by tumor cells and microenvironments to enhance tumor metastasis. That tumor cells usurp pathways critical to the developing embryo is not surprising, as many of the normal developmental programs include processes that are also seen during tumor progression to a metastatic phenotype, including epithelial to mesenchymal transition (EMT), tissue specific morphogenesis, cellular motility and invasion (Fig. 1).

BONE MORPHOGENIC PROTEIN

BMPs, members of the TGF-ß family of signaling proteins, are secreted ligands that signal

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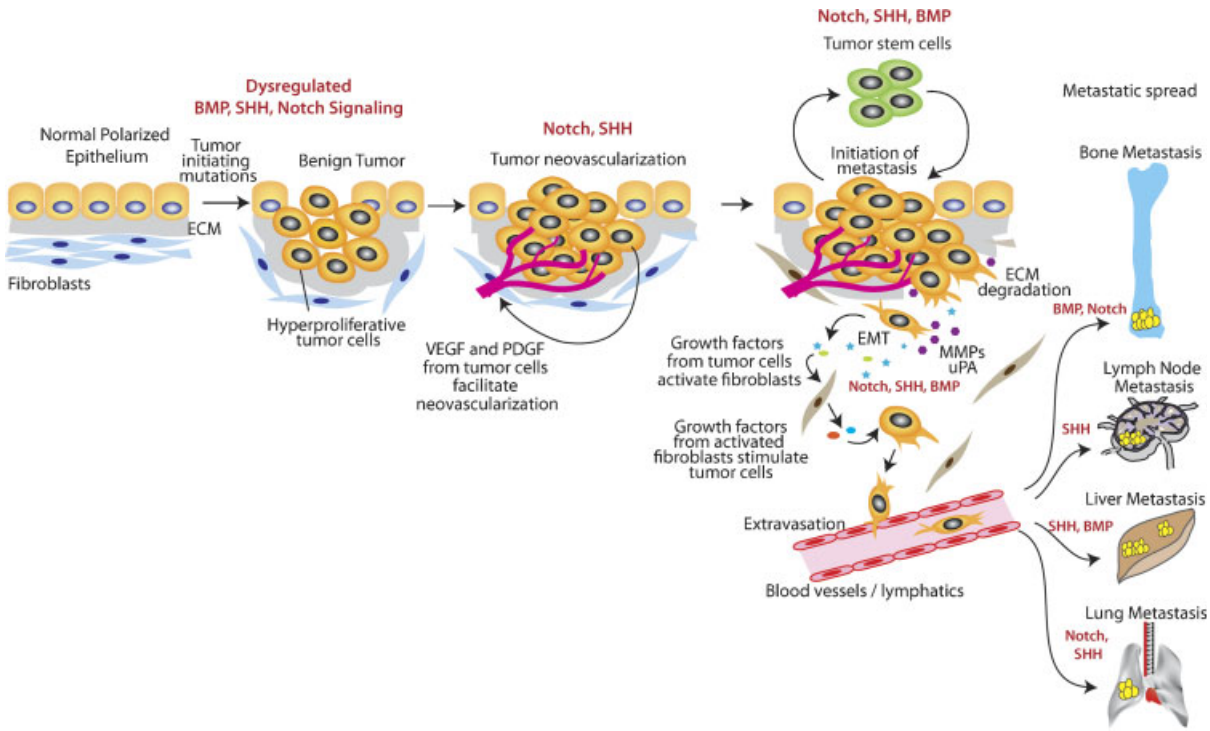

Fig. 1. Developmental pathways in cancer progression and metastasis. Normal polarized epithelial cells gain oncogenic potential through mutations, which leads to loss of apical-basal polarity, unrestricted cell proliferation, and activation of developmental signaling pathways. This results in production of angiogenic factors such as PDGF and VEGF, which facilitates neovascularization, and matrix remodeling factors such as MMPs and urokinase plasminogen activator (uPA), resulting in extracellular matrix degradation and release of invasive tumor cells for metastasis. The tumors also contain a tumor stem cell population that is involved in constant self-renewal and differentiation to epithelial cancer cell populations, and may influence normal tissue based stem cells to produce stroma. Furthermore, epithelial-mesenchymal transition occurs at invasive tumor front, providing a highly motile and invasive phenotype to the tumor cells. Tumor cells also activate the surrounding stromal cells which further enhances oncogenic potential of tumor cells and facilitates metastasis to distant organ environments through vascular/lymphatic systems. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

via both autocrine and paracrine mechanisms to regulate cell proliferation and differentiation. BMP ligands bind to cell surface-associated proteins called bone morphogenic receptors type I and type II (BMPr I and II). There are three known BMP I receptors: type IA and IB BMP receptors and type IA activin receptor [Koenig et al., 1994; ten Dijke et al., 1994]. There are also three known type II receptors: type II-BMPr, type II and IIB activin receptors [Kawabata et al., 1995; Nohno et al., 1995; Rosenzweig et al., 1995; Yamashita et al., 1995]. The receptors are differentially expressed on organs and cell types. The presence of both types I and II receptors is essential to pathway activation. BMP ligand binding facilitates the heteromeric association of the type I and II receptors and receptor activation occurs through the phosphorylation of the type I receptor by the type II receptor [Moustakas and Heldin, 2002]. The type I BMPr propagates a signaling cascade by phosphorylating Smads 1, 5, and 8, which results in the association of these Smads with Smad 4 [Hoodless et al., 1996; Cao and Chen, 2005]. Association with Smad 4 enables the nuclear translocation of these complexes and the transcriptional activation of target genes [Derynck et al., 1998]. The BMP proteins and their receptors contribute to multiple developmental processes including dorsoventral patterning from the neural tube, hematopoesis, cardiac development, skeletal differentiation and skeletal formation. BMP proteins are also important for adult and embryonic stem cell fate and proliferation (Fig. 2).

BMP IN VERTEBRATE DEVELOPMENT

The BMP proteins have been studied extensively in developmental models, which give

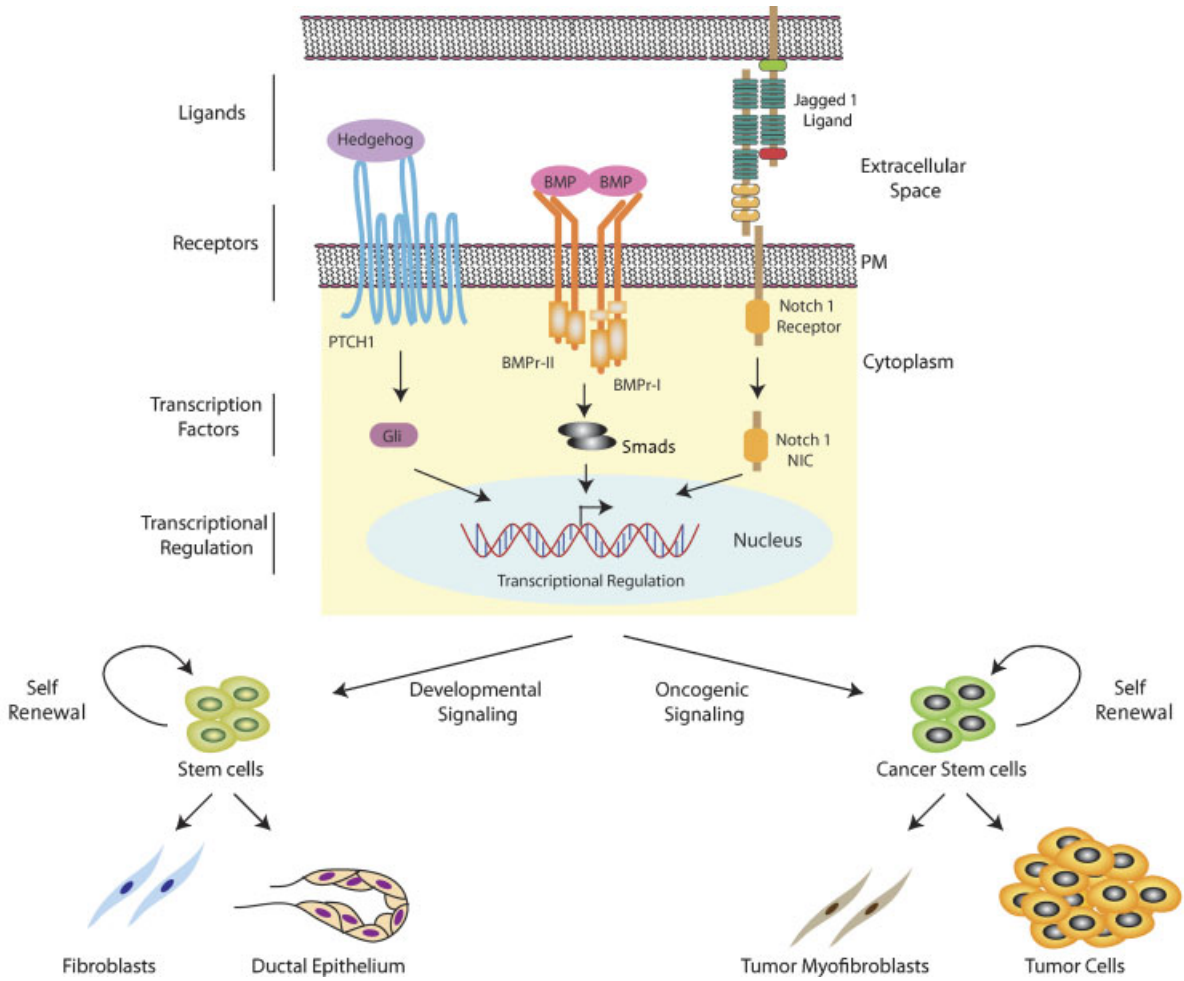

Fig. 2. Common elements in signaling regulation of development and cancer. Developmental signaling cascades such as hedgehog, BMP and Notch are involved in activation of stem cells in development and cancer. Hedgehog, BMP, and Delta bind to their respective receptors Patched, BMPR and Notch, which respectively leads to activation of transcription factors, Gli, Smad and intracellular domain of Notch (ICD, generated by γ-secretase mediated cleavage of Notch receptor in a ligand dependent manner). These transcription factors activate transcriptional events that contribute to self-renewal and differentiation of stem cells. In the case of cancer stem cells, these signaling events enhance the tumorigenic potential by influencing the local organ environment. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

significant insight into the mechanisms tumor cells appropriate to enhance growth and metastasis. *BMP-2* and *BMP-4* knockout mice are non-viable and *BMP-4* knockout mice lack mesodermal differentiation [Winnier et al., 1995; Zhang and Bradley, 1996]. *BMP-2/-4* conditional knockout mice show severe defects in bone development [Cox, 2004]. Similarly, *BMPr-1* deletions in mice show lack of differentiation of chondrocytes and reduction in pre-chondrogenic cells [Yi et al., 2000].

Further insight into the function of BMP proteins has been established by experiments altering the expression of known BMP antagonists, including Smad 6, Tob, Noggin, and Smad ubiquitin regulatory factor, an E3 ligase that regulates the degradation of Smads 1 and 5. Experiments in which these BMP antagonists are overexpressed show deleterious phenotypes in mice. Noggin acts as an antagonist through its association with the BMP ligands, inhibiting their association with the BMPr [Groppe et al., 2002]. Transgenic mice that express the Noggin transgene develop osteoporosis/osteopenia and show significant loss of bone density, bone volume, and bone formation rates [Devlin et al., 2003]. Tob antagonizes BMP signaling through its association with Smads 1 and 5 [Yoshida et al., 2000]. In *Tob* knockout mice, BMP-2 signaling is increased, which

enhances osteoblast proliferation and differentiation culminating in an increase in bone length and volume [Yoshida et al., 2000]. Smad 6 is an antagonist that binds to the BMPrI receptor to interrupt the phosphorylation of Smads 1 and 5 by the BMPr-I receptor [Imamura et al., 1997]. *Smad 6* knockout mice develop hyperplasia of the heart and other cardiovascular abnormalities [Galvin et al., 2000]. Thus, the *BMP* proteins are regulators of cellular growth and differentiation and are potent regulators of bone formation and development of other organs.

BMP proteins are also critical for stem cell fate and renewal, implicating a role in the renewal and proliferation of cancer stem cells. BMPs, while detrimental to neuronal stem cell differentiation, are critical to maintenance of undifferentiation and self-renewal in mouse embryonic stem (mES) cells [Munoz-Sanjuan et al., 2002; Ying et al., 2003]. BMPs have been shown to have a different effect in humans. BMPs have been implicated in regulating the differentiation of human ES (hES) cells. BMP proteins induce the differentiation of hES cells into cells of endoderm lineage and inhibitors of BMP such as Noggin inhibit the differentiation of hES cells [Pera et al., 2004]. Stem cells isolated from the neural crest (NCSC) in both mice and humans and stimulated with BMP-2 begin to express Achaete–Scute complex homologue 1 (Mash-1), which is an early marker of neurogenesis [Shah et al., 1996], and these stem cells ultimately differentiate into autonomous neurons [Shah et al., 1996]. These data implicate BMP in the maintenance of undifferentiation in stem cells, but also in the differentiation of specific lineages of certain stem cells and determination of cell fate.

BMP IN CANCER METASTASIS

Dysregulation of BMP protein expression has been documented in several types of cancer. BMP-4, -6, and -7 are expressed in prostatic adenocarcinomas with known skeletal metastasis [Hamdy et al., 1997; Masuda et al., 2003]. In oral epithelium, BMP-2, -4, and -5 have been observed in high-risk malignant and metastatic lesions [Jin et al., 2001]. Other BMPs are upregulated in gastric, breast and colon cancers and melanoma cell lines. Recently, BMP-4 protein and mRNA was shown to be overexpressed in advanced stages of colorectal cancer and in highly invasive epithelium, but absent in the normal colonic mucosa [Deng et al., 2007a]. The levels of BMP receptors (BMPr-1A and BMPr-II) were similar in all stages of colorectal cancer. The effects of BMP expression were further analyzed by overexpressing the protein in HCT116 cells, which protected these cells from serum starvation-induced apoptosis and increased their motility and invasion activities. Invasion was inhibited with the BMP antagonist, noggin [Deng et al., 2007a]. These data implicate BMP-4 in the metastasis of colorectal cancer and also in the selection of highly metastatic cancer cells.

BMP-2 has been implicated in the metastasis of breast cancer cell lines. mRNA levels of BMP-2 and BMPR's were higher in metastatic human breast cancer cells than less metastatic cancer cells [Arnold et al., 1999]. Breast cancer cell lines were shown to migrate towards a BMP-2 source and BMP-2-expressing MCF-7 cells were shown to invade and migrate through matrigel with enhanced migratory capabilities through expression of BCSG1, a metastasis-related gene [Clement et al., 2005]. When examined in a mouse xenograft model, the BMP-2 expressing MCF-7 tumors showed enhanced tumor formation and vasculature in an estrogen-independent manner [Clement et al., 2005].

Hepatocyte growth factor (HGF) upregulated the expression of BMPr-IB and II in prostate cancer cell lines. HGF-stimulated xenografts showed upregulated expression of these receptors, implicating HGF-mediated regulation of the pathway in prostate cancer metastasis [Ye et al., 2007]. BMP-2 and -4 enhanced the migration and invasion of a highly aggressive prostate cancer cell line, PC-3 both in vitro and in vivo and BMP-2 expression was correlated with a high rate of osteolytic lesion formation by the PC-3 cell line [Feeley et al., 2006].

Individual BMP proteins have also been associated with EMT. In cardiac cushion development, BMP-2 has been associated with EMT [Ma et al., 2005] and BMP-4 has been shown to induce EMT in human ovarian cancer cells [Theriault et al., 2007]. BMP-7, however, has been shown to antagonize TGF-β-induced EMT in renal cells and in renal cell injury [Zeisberg et al., 2003].

The effects of BMP-2 and -4 proteins on proliferation, motility, and invasion of tumor

cells are complemented by findings that they enhance differentiation and proliferation of progenitor cells in embryonic and adult development. Moreover, BMP-2 has been shown to function in stem cell renewal and differentiation. Highly metastatic and invasive cancer cells express this protein in multiple types of cancer, which implicates BMP-2 as a regulator of cancer stem cell proliferation and maintenance. BMP proteins may help select for more aggressive tumor cells through an autocrine signaling pathway, by stimulating the progenitors that colonize metastases.

BMP proteins may also be regulators of the tumor microenvironment. BMP proteins, secreted from tumor cells, may signal in a paracrine manner to create a reactive stroma through the activation of tumor-associated myofibroblasts. Tumor-associated myofibroblasts are known enhancers of tumor cell growth and metastasis.

BMP proteins may have profound effects on adult tissues once secreted in local environments, including the inhibition of progenitor cell differentiation in tissues such as the colon or liver, which are constantly regenerating new cells and tissues to maintain homeostasis. Recently, a number of papers have suggested expression of BMP-2 and -4 can downregulate the expression of matrix metalloproteases (MMP)-1, -9, -3, and -13 [Takiguchi et al., 1998; Kumagai et al., 2006; Otto et al., 2007], suggesting that the invasive role of BMPs could be dependent on the tissue type. Once the metastatic cells reach their target metastatic niche, the continued expression of BMP in the context of a different microenvironment may actually reduce the levels of MMPs to enhance colonization and facilitate metastasis. Another possibility is that BMP proteins function as chemoattractant molecules. BMP proteins secreted from bone may recruit highly metastatic tumor cells that express the BMP receptors to "pre-neoplastic" niches.

Many questions remain with respect to the biology of BMP proteins in cancer, given that there are currently over 20 identified BMP signaling ligands. Recent data describe homodimeric associations of BMP receptors and highlight pathway activation through the p38 MAK pathway, which implicates downstream mediators other than Smad signal transducers [Deng et al., 2007b]. Given their roles in primary tumor growth, extravasation, and metastasis, the BMP proteins present many avenues for developing therapies and new diagnostic procedures.

NOTCH

Notch signaling is critical for cell–cell communication and regulates a broad spectrum of cell fate specifications during embryonic development and in the adult organism. In development, Notch is instrumental in regulating processes such as neurogenesis, somitogenesis and angiogenesis—reviewed in Bolos et al. [2007]. Dysregulation of the Notch signaling pathway has been implicated in cancer. There are four mammalian Notch proteins (*Notch 1–4*), which are transmembrane protein receptors. The *Notch* genes encode transmembrane receptors, which contain a large extracellular domain, composed of a variable number of epidermal growth factor (EGF)-like repeats and an intracellular signaling domain (NIC) [Artavanis-Tsakonas et al., 1999]. The NIC domain consists of six ankyrin/cdc10 motifs and nuclear localization signals. Notch receptors interact through their extracellular domain with other membrane-associated ligands. These other membrane-bound ligands are of the Delta and Serrate/Jagged families [reviewed in Artavanis-Tsakonas et al., 1999]. Ligand interaction with the extracellular region of Notch facilitates the cleavage of Notch by a γ-secretase activity, yielding the intracellular NIC signaling domain. NIC then translocates to the nucleus and associates with the RBPJK/CBF1/Su(H) transcription factor to induce expression of target genes [Kopan, 2002].

NOTCH SIGNALING IN DEVELOPMENT

Our understanding of the role of Notch signaling in the central nervous system (CNS) provides insight into Notch's potential to drive tumor progression and metastasis. In neurogenesis, Notch is a mediator of cellular differentiation status. As a cell commits to neuronal differentiation, it expresses more Delta (ligand for Notch). A progenitor cell with elevated levels of Delta becomes a neuron and sends inhibitory signals to other progenitor cells to maintain their undifferentiated status, which inhibits them from expressing Delta

[Lewis, 1998]. The ability of Notch to control differentiation and maturation through this process is termed lateral inhibition [Cabrera, 1990].

Notch signaling has also been shown to promote angiogenesis and EMT [Timmerman et al., 2004]. The heart is one developmental system in which these functions for Notch have been extensively studied. The heart forms from the cardiac mesoderm early in development and is the first organ system to form during vertebrate embryogenesis. Proper development of the cardiovascular system depends on the coordinated development of valves and vascularization. Notch1 and Notch4, along with Delta4 and Jagged1 are expressed in the cardiac and vascular systems during embryonic development and contribute significantly to their ordered development [Uyttendaele et al., 1996; Krebs et al., 2000]. Downstream effectors of Notch signaling and target genes such as *RBPJk*, *HRT1*, and *HRT2* have also been identified in cardiac and vascular development [Timmerman et al., 2004]. Mice that are *Notch1* and *Notch4* double mutants have apparently normal vasculogenesis; however, they show impaired angiogenesis in the embryo proper and placenta [Krebs et al., 2000], implicating a significant role for Notch in angiogenesis. In a separate, in vitro model, Jagged-Notch signaling has been shown to promote fibroblast growth factor-induced endothelial cell migration and in vitro blood vessel formation [Zimrin et al., 1996]. In the process of EMT, Notch has a significant role during cardiac development. Notch signaling is important in endocardial maturation at E9.5 in mice, where the endocardial cells undergo Notch-induced EMT to invade through a thick extracellular matrix called the cardiac jelly and form cardiac cushions. *Notch1* mutants have a collapsed endocardium and show an absence of mesenchymal cells in the cardiac cushions [Timmerman et al., 2004]. Thus, Notch is also a critical mediator of both angiogenesis and EMT.

NOTCH SIGNALING IN CANCER METASTASIS

Notch signaling has been classified as either oncogenic or tumor-suppressive depending on the cell type, specific type of mutation within the Notch pathway, the timing in the context of transformation and metastasis and the tissue context [Maillard and Pear, 2003; Radtke and Raj, 2003]. Notch has distinct roles in different organs and tissues and thus, the ability of Notch to drive or suppress transformation and metastasis is dependent on the tissue and organ site in which it is expressed. If Notch is required for stem cell maintenance, then pathway activation is associated with an oncogenic function. However, when pathway activation is critical for differentiation, then the pathway has tumor-suppressive capability.

Notch has been recently reported to activate NFκB in pancreatic cancer [Wang et al., 2006]. In experiments conducted on pancreatic cancer cell lines, inhibition of Notch1 decreased NFκB-DNA binding potential and decreased the expression levels of MMP-9, one of the most significant MMP involved in the extravasion of pancreatic tumor cells from their epithelium. This study also showed that downregulating Notch1 in these cells further downregulated expression of VEGF, COX-2, and survivin, all signaling mechanisms critical to invasion and metastasis in pancreatic cancer. The resultant phenotype was loss of invasive potential by pancreatic cancer cell lines [Wang et al., 2006]. These data implicated Notch1 as a regulator of NFκB-DNA binding, which affected the regulation of specific genes critical for metastasis. Likewise, activated Notch signaling induced invasive phenotypes in breast mammary epithelial cells and in keratinocytes. Notch signaling has been shown to enhance metastatic properties of primary melanoma cells through effects on β-catenin signaling [Balint et al., 2005].

In Prostate cancer, the Jagged ligand is more highly expressed in metastatic prostate cancer when compared to localized prostate cancer or benign prostatic tissues [Santagata et al., 2004]. This finding was important as Notch1 leads to an increase in osteoblast differentiation and thus may select for metastatic prostate cancer cells that are able to colonize the bone. Moreover, *Notch1* expression was enhanced 4–5 times in osteoblastic skeletal prostate cancer cell lines compared to non-skeletal metastatic cell lines. Both Notch and ERK phosphorylation were important for metastatic cells to acquire "osteoblast-like" properties and to establish phenotypes that enhanced their survival in the metastatic bone tissue [Zayzafoon et al., 2004].

NOTCH AND THE "PRE-NEOPLASTIC" NICHE

Recently, Notch signaling was implicated in the ability of *Drosophila* germline stem cells to signal to their surrounding niche to enhance stem cell renewal and long-term survival within the niche [Ward et al., 2006]. Germline stem cells expressed the receptors Delta and Serrate, which enabled them to signal to the somatic cells of the niche through the Notch receptor. These signaling interactions facilitated proper regulation of the TGF-β and Piwi pathways, which are required to maintain a functional niche and support stem cell maintenance and division [Ward et al., 2006]. These results provide intriguing insight into the potential role of Notch signaling in facilitating the interactions of tumor stem cells and their many proposed niches. Depending on the tissue of origin and the route of spread, a regulatory mechanism enabling certain tumor stem cells from a given tissue to survive in specific niche environments is highly consistent with the "seed and soil" hypothesis of metastasis [Fidler, 2003]. Notch signaling is regulated by timing and signal strength. Thus, the number of ligands expressed on a tumor stem cell will directly affect niche interactions, and the net effect is codependent on the receptors expressed on the somatic cells in the niche tissue. Further investigation of the contribution of Notch to tumor stem cell niche during metastasis is warranted.

SONIC HEDGEHOG

The hedgehog family of signaling proteins are secreted proteins that signal through both autocrine and paracrine mechanisms to control cell proliferation, differentiation, and morphology [Ingham and McMahon, 2001]. There are three known hedgehog ligands, Sonic (Shh), Indian (Ihh), and Desert (Dhh). Shh is more closely related to Ihh, while Dhh is more closely related to the hedgehog of *Drosophila*. The hedgehog proteins exert their function by binding to a 12-pass transmembrane protein called Patched (PTCH) [Pepinsky et al., 2000]. This interaction relieves the inhibitory affect of PTCH on a serpentine protein called Smoothened (SMO) [Murone et al., 1999]. SMO is then hyperphosphorylated and has been recently shown to localize to primary cilia [Corbit et al., 2005]. This pathway ultimately concludes with the activation and repression of target genes through the Gli family of transcription factors. In mammals, there are three Gli transcription factors (*Gli-1*, *-2*, *-3*) that regulate the transcription of target genes. Gli3 has been shown to be the transcriptional repressor that inhibits the transcription of target genes, maintaining the pathway in an inactive state in the absence of hedgehog ligand stimulation. Gli2 has been shown to contain the activating function and translocates to the nucleus to activate transcription of target genes downstream of SMO phosphorylation [Lipinski et al., 2006]. In mouse embryonic fibroblasts, Gli2 translocates to the nucleus in response to SHH stimulation. Gli1 expression is then elevated [Lipinski et al., 2006].

The hedgehog signaling proteins are key mediators of embryonic development. While all the hedgehog ligands play some role in development, SHH will remain the focus of this review. Throughout embryonic development, SHH is expressed in the notochord, the floorplate of the neural tube, the brain, the zone of polarizing activity in the developing limbs and the gut [Roelink et al., 1994; Odenthal et al., 2000]. SHH specifically functions in many different ways to contribute to the patterning and formation of a developing embryo. To influence patterning in the embryo, SHH, secreted from the cell from which it is synthesized, elicits different effects in a concentration-dependent manner along a target range to affect proliferation or differentiation in target cells [Ingham, 1998]. In the development of the CNS, the long-range morphogenic properties of SHH signaling are identified as the protein is secreted from the ventral neural tube and controls the levels of Gli transcription factors. SHH, through paracrine signaling, controls the levels of Gli transcription factors and can influence differentiation of neuronal subtypes and control proliferation and survival of progenitor cells [Cayuso et al., 2006]. In development of the gut, SHH is synthesized in the developing gut endoderm, but is excluded from the areas that give rise to the pancreas [Apelqvist et al., 1997; Hebrok et al., 1998]. Furthermore, ectopic expression of SHH excludes development of the pancreas and instead results in epithelial-mesenchymal metaplasia and development of the duodenal mesoderm [Apelqvist et al., 1997; Hebrok et al., 1998; Kawahira et al., 2005]. Temporal and

spatial regulation of SHH signaling are critical to the proper development and patterning of many organ systems in both *Drosophila* and in mammals.

SHH is a mediator of angiogenesis and has been shown to induce vessel formation in endothelial cells [Pola et al., 2001]. SHH was also shown to induce the expression of angiopoietins I and II and the family of VEGF signaling proteins from mesenchymal cells, highlighting the significance of tumor-associated fibroblasts in combination with SHH signaling to mediate blood vessel formation [Kanda et al., 2003].

SHH AS A REGULATOR OF METASTASIS

Mutations in the SHH pathway genes during development lead to a variety of embryonic defects and diseases [Pasca di Magliano and Hebrok, 2003]. Mutations in this pathway in the adult are associated with increases in cellular proliferation, transformation, and ultimately cancer. An oncogenic form of SHH has been identified in basal cell carcinoma and SHH is misregulated in pancreatic adenocarcinoma, prostate adenocarcinoma, esophageal and stomach cancer and non-small cell carcinoma—reviewed in Pasca di Magliano and Hebrok [2003]. Misregulated SHH signaling contributes to mechanisms whereby these cancers use both autocrine and paracrine signaling to affect proliferation and differentiation of their surrounding environment. Inhibition of SHH signaling has been shown to reduce tumor burden and metastasis in both prostate and pancreatic adenocarinomas [Sanchez et al., 2004; Feldmann et al., 2007]. Recently, pancreatic cancer stem cells were shown to express high levels of SHH [Li et al., 2007], which is interesting given the implications for SHH in adult stem cell renewal, in pancreatic ductal progenitor cells and also in adult hair follicle stem cells [Katoh and Katoh, 2006]. SHH has also been shown to affect EMT and disruption of SHH signaling by the inhibitor cyclopamine inhibited EMT in pancreatic cancer cell lines [Hay, 1995; Feldmann et al., 2007]. In esophageal squamous cell carcinoma, Gli1 expression has been associated with lymphatic metastasis [Kawahira et al., 2005] and inhibition of SHH pathway using the inhibitor cyclopamine reduced cell growth and motility.

CONCLUSION AND PERSPECTIVE

Highly aggressive tumor cells have been shown to share many of the characteristics of embryonic progenitor cells. Many pathways which are imperative to the proper development of a human embryo have also been shown to be active in cancer formation and metastasis. The processes by which these highly aggressive tumor cells usurp developmental pathways to enhance proliferation rates and metastasis should be further investigated. Notch, BMP and SHH are examples of pathways that are essential to embryonic development and patterning and are also used by tumor cells to promote their survival and metastasis. Their involvement in embryonic development suggest that Notch, BMP, and SHH signaling, when activated either individually or in combination in cancer cells, facilitate the survival of tumor stem/progenitor cells. These pathways may also function in concert to orchestrate embryonic-type microenvironments. There is an imminent need to address questions that remain regarding these pathways and their effects on regulating metastasis to enable the discovery of novel therapeutic targets.

The sections described in this review highlighted instances where the pathways individually were shown to affect metastasis. Is there evidence for these pathways acting together and what are the implications for metastatic tumor cells during remodeling of their microenvironments? Do the pathways individually or in combination affect the organ sites to which certain tumor cell types metastasize? BMP is known to affect bone metastasis by prostate tumor cells and SHH is also known to be secreted by prostate cancer cells. However, BMP-2, SHH, and Notch are all activated or expressed in pancreatic cancer, an aggressive adenocarcinoma that is highly metastatic, yet one that does not characteristically metastasize to the bone. Therefore, the activation of these pathways alone does not account for organ-specificity in the context of metastasis, yet in certain cancers, their expression is essential for organ-specific metastasis. The composite effects of activating multiple pathways should be explored in future studies.

In the context of both pancreatic and prostate cancers, there is also a highly reactive stroma and thus, the possibility remains that BMP, SHH, and

Notch signal and interact in a paracrine manner to orchestrate a more embryonic-type environment. This embryonic microenvironment not only affects primary tumor EMT and angiogenesis, but also alters the microenvironment within metastatic organs and may enables a more "plastic" tumor stem cell to survive in an organ outside the pancreas.

Another important question is the role of these signaling molecules in altering the tumor microenvironment and setting up pre-metastatic niches. The pre-metastatic niches have been hypothesized to arise when tumor cells in circulation reach an organ and send signals to create an environment that facilitates the colonization of other tumor cells, thus giving rise to metastasis. Given their distinct roles in patterning, any of these proteins may alter their surrounding environment in such a manner, as to enhance the colonization of cells and drive metastasis.

There is also the question of what ultimately is the cause of death in a cancer patient. Each of the pathways highlighted in this review has profound effects on multiple tissues and organs throughout development, ranging from the neurologic system, cardiovascular function, development of the gut and also the skeletal system. Is it possible that circulating tumor cells and enhanced expression of these morphogenic proteins from tumor cells ultimately compromises the function of these organ systems, even in the absence of advanced metastasis within the organ? Could these deleterious effects lead to the ultimate demise of the cancer patient? If so, then methods to target these pathways, even if patients with advanced disease, may help prolong life or enhance quality of life, through the sustained ability of other organs to function.

In summary, our knowledge of the roles of SHH, BMP, and Notch in development are increasing, but much remains to be discovered regarding how these pathways act as regulators of metastasis. We have proposed and discussed hypotheses for how embryonic pathways may contribute to tumor stem cell maintenance, facilitate tumor–stroma interactions, and influence normal cell function at metastatic sites. A better understanding of the mechanisms whereby these pathways contribute to tumor progression and metastasis will ultimately lead to new and improved therapies for multiple cancers.

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