Review

Periostin: Novel diagnostic and therapeutic target for cancer

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Summary. Periostin is a secreted protein that shares a structural homology to the axon guidance protein fasciclin I (FAS1) in insects and was originally named as osteoblast-specific factor-2 (Osf2). Periostin is particularly highly homologus to ßig-h3, which promotes cell adhesion and spreading of fibroblasts. It has recently been reported that Periostin was frequently overexpressed in various types of human cancers. Although the detailed function of Periostin is still unclear, Periostin-integrin interaction through FAS1 domain is thought to be involved in tumor development. In addition, Periostin stimulates metastatic growth by promoting cancer cell survival, invasion and angiogenesis. Therefore, Periostin can be a useful marker to predict the behavior of cancer. This review summarizes the recent understanding of Periostin roles in tumor development and speculates on the usefulness of Periostin as a therapeutic and diagnostic target for cancer.

Key words: Periostin, Invasion, Metastasis, Angiogenesis, Cancer

Periostin

Periostin, originally named as osteoblast-specific factor-2 (Osf2) (genebank D13664), first identified in bone, was implicated in regulating adhesion and differentiation of osteoblasts (Horiuchi et al., 1999; Litvin et al., 2004). Periostin is assigned to one family based on its homology to fasciclin I (FAS1) identified in

insects. Proteins that share homology with FAS1 include ßig-h3, stablin I and II, MBP-70, Algal-CAM, Periostin, and Periostin-like-factor (PLF) (Zinn et al., 1988; Terasaka et al., 1989; Skonier et al., 1992; Takeshita et al., 1993; Huber and Sumper, 1994; Horiuchi et al., 1999; Litvin et al., 2004).

Takeshita et al. cloned mouse *POSTN*, which they designated Osf2. By screening human placenta and osteosarcoma cDNA libraries with mouse *POSTN* as a probe, they cloned 2 variants of human *POSTN* (Takeshita et al., 1993). One variant encodes a deduced 779-amino acid protein with an apparent molecular mass of 87.0 kD, and the other encodes a deduced 836-amino acid protein with an apparent molecular mass of 93.3 kD. Gillan et al. also identified a Periostin EST clone encoding a deduced 782-amino acid protein (Gillan et al., 2002). Approximately 90 kDa Periostin has an NH_2-terminal secretory signal peptide, followed by a cysteine-rich domain, four internal homologous repeats and a COOH-terminal hydrophilic domain (Takeshita et al., 1993; Horiuchi et al., 1999) (Fig. 1). The four internal repeats region of Periostin share a homology with an axon guidance protein FAS1, containing sequences that allow binding integrins and glycosaminoglycans *in vivo* (Elkins et al., 1990). Moreover, in N-terminus, Periostin has EMI domain, which is a small cystein-rich module of ~75 amino acids (Fig. 1). The EMI domain was first named after its presence in proteins of the EMILIN family and is associated with other domains, such as C1q, laminin-type EGF-like, FN3, WAP, ZP or FAS1 (Doliana et al., 2000; Callebaut et al., 2003). Mouse and human *POSTN* share 89.2% amino acid identity overall and 90.1% identity in their mature forms. Mouse periostin is located

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Abbreviations: FAS1, fascilin I; Osf2, osteoblast-spècific factor-2; PLF, Periostin-like-factor; EC, extracellular matrix; EMT, epithelial mesenchymal transformation; VEGF, Vascular endothelial growth factor; TGF-ß1, transforming growth factor ß1.

on chromosome3 and human periostin is on chromosome13q.

Periostin expression in cancer

By RNA dot blot analysis, Periostin expression was observed in a wide range of normal adult tissues, including aorta, stomach, lower gastrointestinal tract, placenta, uterus, and breast (Gillan et al., 2002). Periostin protein expression is observed in normal adult tissues including adrenal glands, lung, thyroid, stomach, colon, vagina, ovary, testis and prostate by Western blot analysis (Tai et al., 2005). Moreover, Periostin is highly expressed in developing and mature heart valves (Kruzynska-Frejtag et al., 2001), under pressure or volume overload in the adult heart (Stanton et al., 2000; Katsuragi et al., 2004), in developing teeth (Kruzynska-Frejtag et al., 2004), in skeletal muscle after injury (Goetsch et al., 2003), and in pulmonary aortic smooth muscle cells in response to hypoxia (Li et al., 2004). Secreted Periostin by epithelial ovarian cancer cells, but not normal ovarian epithelial cells is detected in culture medium (Gillan et al., 2002). They identified multiple protein bands of about 90 kD, as well as a band of about 170 kD, which may represent a covalently linked multimer (Gillan et al., 2002). Recently, it has been reported that Periostin is frequently overexpressed in various cancers as described below. Thus, Periostin

expression is ubiquitous, highly expressed in the embryonic periosteum, cardiac valves, placenta, periodontal ligament and many adult cancerous tissues.

Periostin was found to be overexpressed in various types of human cancer including neuroblastoma (Sasaki et al., 2002), head and neck cancer (Kudo et al., 2006; Siriwardena et al., 2006), nasopharygeal carcinoma (Chang et al., 2005), thyroid carcinoma (Fluge et al., 2006), non-small cell lung carcinoma (Sasaki et al., 2001a), breast cancer (Shao et al., 2004; Grigoriadis et al., 2006), colon cancer (Bao et al., 2004; Tai et al., 2005), pancreatic ductal adenocarcinoma (Baril et al., 2006), and ovarian cancer (Gillan et al., 2002). Interestingly, elevated levels of Periostin have been detected in sera of patients with thymoma (Sasaki et al., 2001b), non-small cell lung carcinoma (Sasaki et al., 2001c), breast cancer (Sasaki et al., 2003) and pancreatic
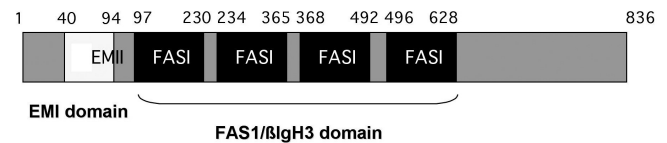

Fig. 1. Schematic domain structure of Periostin is shown. The position of EMI domain and four FAS1/ßIgH3 domain is indicated.

Table 1.

Cancer type/Periostin expression	Periostin function	Reference
Non-small cell lung cancer		
Up-regulation in tissues and serum	Correlation with clinical stage and survival	Sasaki et al., 2001a and 2001c
Thymoma		
Up-regulation in serum	Correlation with clinical stage	Sasaki et al., 2001b
Neuroblastoma		
Up-regulation in tissues	Correlation with clinical stage	Sasaki et al., 2002
Ovarian cancer		
Up-regulation in tissues and serum	Cell motility	Gillan et al., 2002
Cancer cell line (bladder and osteosarcoma)		
Down-regulation	Suppress anchorage independent growth	Yoshioka et al., 2002
Breast cancer		
Up-regulation in serum	Corelation with bone metastasis	Sasaki et al., 2003
Up-regulation in tissues	In vivo tumour growth and angiogenesis	Shao et al., 2004
Up-regulation in tissues	Correlation with survival	Grigoriadis et al., 2006
Colon cancer		
Up-regulation in tissues	Cellular survival, angiogenesis and metastasis	Bao et al., 2004
Up-regulation in tissues	Cell proliferation and cellular survival	Tai et al., 2005
Nasopharingeal cancer		
Up-regulation in tissues	Correlation with TGF-ß expression	Chang et al., 2005
Bladder cancer		
Down-regulation	Suppress invasion and metastasis	Kim et al., 2005
Head and neck cancer including oral cancer		
Up-regulation in tissues	Invasion, anchorage-independent growth and metastasis	Kudo et al., 2006
Up-regulation in tissues	Invasion, angiogenesis and metastasis	Siriwardena et al., 2006
Cancer cell line (293T)		
Not determined	Invasion, EMT and metastasis	Yan and Shao, 2006
Pancreas cancer		
Up-regulation in tissues	Invasion and suppression of hypoxia-induced cell death	Baril et al., 2006

ductal adenocarcinoma (Baril et al., 2006). Periostin was also detected in ascites from ovarian cancer patients (Gillan et al., 2002). Importantly, Periostin expression is well correlated with its malignant behavior such as invasion, metastasis and/or poor survival in neuroblastoma (Sasaki et al., 2002), head and neck cancer (Kudo et al., 2006; Siriwardena et al., 2006), non-small cell lung carcinoma (Sasaki et al., 2001a), breast cancer (Grigoriadis et al., 2006) and colon cancer (Bao et al., 2004). Although these reports implicate Periostin in tumour spread, the functional role of this protein is poorly described (Table 1). On the other hand, Kim et al. reported that Priostin overexpression suppressed the invasiveness and metastasis of tumour cells in bladder carcinoma (Kim et al., 2005). Moreover, Yoshioka et al. also found that Periostin overexpression suppressed anchorage-independent growth in bladder cancer and osteosarcoma cell lines (Yoshioka et al., 2002). Although Priostin may play an important role for tumor progression in various types of cancer, Periostin may function as a supresor of tumor progression in some types of cancer such as bladder cancer and osteosarcoma. To prove this, investigation of Periostin expression is required in a larger cohort of various cancer cases.

Periostin function in cancer

Recently, Periostin was identified as an invasion and metastasis related gene by differential cDNA display analysis among normal colon tissue, primary colon cancer and metastatic tumor in the liver (Bao et al., 2004), and by comparing the gene expression profiles of the parent oral cancer cells and highly invasive clones (Kudo et al., 2006). In the presence of Periostin, tumor cells enhance invasive activity by forming fewer stress fibers and increasing the motility of cells (Gillan et al., 2002). *In vivo* studies by xenograft assays showed that Periostin enhances tumour growth, cancer cell survival, angiogenesis and metastasis compared to Periostin non-expressing cells (Bao, 2004; Kudo et al., 2006; Yan and Shao, 2006). Thus Periostin plays important roles for tumor progression including invasion, angiogenesis, cellular survival, and metastasis (Fig. 2) (Table 1). The detailed function of Periostin in tumor progression is described as follows;

FAS1 domain and integrin

Periostin has FAS1 domains. In mammals, there are two secretory proteins containing FAS1 domains, Periostin and ßig-h3. FAS1 of ßig-h3 bears motifs interacting with integrins, α3ß1 and αvß5 (Kim et al., 2000, 2002), and mediates endothelial cell adhesion and migration via integrin αvß3 (Nam et al., 2003). In addition, ßig-h3 contains an RGD motif near the COOH terminus, but this integrin recognition site can be deleted without affecting cell adhesion (Ohno et al., 1999). Although Periostin does not contain an RGD motif, ßig-

h3 does not contain the sequence homologous to the C-terminal hydrophilic domain in Periostin, suggesting that functional differences may exist between the two proteins during tumor development.

The adhesion of epithelial cells to the ECM involves both integrin-dependent and independent mechanisms. Integrins are transmembrane heterodimeric receptors involved in both cell-cell and cell-ECM interactions (Hynes, 1992). The functions of integrins are not limited to cell adhesion, but also involve activation of cytosolic signaling cascades to mediate cell proliferation, cell survival, and cell migration (Schwartz et al., 1995; Lafrenie and Yamada, 1996). Integrin expression is frequently altered in cancer cells (Varner and Cheresh, 1996; Mizejewski, 1999), which together with the changes in the ECM composition alters the adhesion and
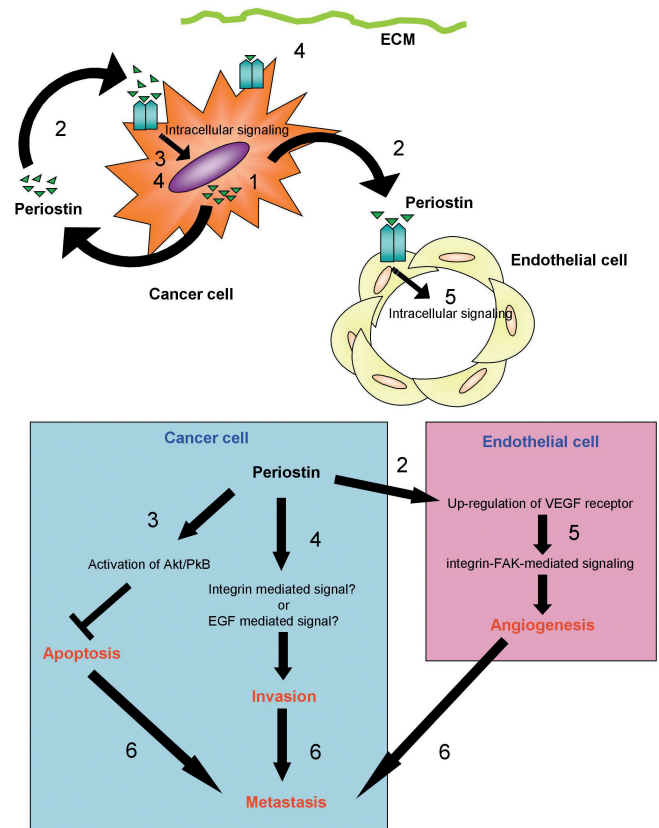

Fig. 2. Schematic model of Periostin function in cancer. Invasion, cellular survival and angiogenesis promoted by Periostin lead to metastasis of cancer cells through the following steps. 1) Cancer cells with high expression of Periostin secrete Periostin. 2) Secreted Periostin binds to integrins in cancer cells and endothelial cells. 3) Periostin induced cellular survival through the activation of Akt/PKB pathway via αvß3 integrin in cancer cells. 4) Interaction between Periostin and integrins promotes invasion through the inhibition of interaction between integrins and ECM and/or activation of intra-cellular signal. 5) Interaction between Periostin and integrins promotes angiogenesis in endothelial cells. 6) Invasion, cellular survival and angiogenesis leads to metastasis.

motility of cancer cells. Purified recombinant Periostin supported adhesion of ovarian epithelial cells, and adhesion was inhibited by antibodies against αvß3 or αvß5 integrins, but not by antibodies against ß1 integrin, indicating that Periostin is a ligand of integrins αvß3 and αvß5 in ovarian cancer cells (Gillan et al., 2002). A similar result is shown in breast, colon and oral cancer cells (Bao et al., 2004; Shao et al., 2004; Kudo et al., 2006). In pancreas cancer cells, α6ß4 integrin complex acts as the cell receptor of Periostin and this interaction promotes migration through focal adhesion kinase (FAK) phosphorylation (Baril et al., 2006). This selective coordination of inputs from different integrins largely depends on the cell types that inherently express distinct membrane receptors. Taken together, these data strongly suggest that Periostin binds to integrins and that this interaction may be involved in tumor development.

It is well known that integrin mediates cell-extracellular matrix (ECM) interaction and that integrin-mediated adhesion regulates a variety of intracellular events (Meredith and Schwartz, 1997). Periostin transfected cells showed morphological changes as well as an increase in the expression of mesenchymal markers such as vimentin and fibronectin, suggesting that Periostin induces cell invasive activity through epithelial mesenchymal transformation (EMT) (Yan and Shao, 2006). The adhesion of epithelial cells to the ECM involves both integrin-dependent and independent mechanisms. The functions of integrins are not limited to

cell adhesion, but also involve activation of cytosolic signaling cascades to mediate cell proliferation, cell survival, and cell migration (Schwartz et al., 1995; Lafrenie and Yamada, 1996). Integrin expression is frequently altered in cancer cells (Varner and Cheresh, 1996; Mizejewski, 1999), which together with the changes in the ECM composition alters the adhesion and motility of cancer calls. These findings suggest that Periostin-integrin interaction may inhibit the ECM-integrin interaction and trigger the intracellular signaling and activation of certain genes that are involved in tumor progression.

Invasion

Progression from a solid tumor to an invasive tumor is a major prerequisite for metastasis and involves changes in both cell morphology and motility (Friedl and Wolf, 2003; Yokota, 2000). We previously established an oral cancer cell line from a metastatic lymph node (Kudo et al., 2003) and isolated highly invasive clones from this cell line by using *in vitro* invasion assay method (Kudo et al., 2004). Then, we compared the transcriptional profile of the parent oral cancer cell and a highly invasive clone by microarray analysis in order to identify the genes that differ in their expression (Kudo et al., 2006). We identified Periostin as the gene demonstrating the highest fold change expression in the invasive clone. In fact, Periostin overexpressing oral
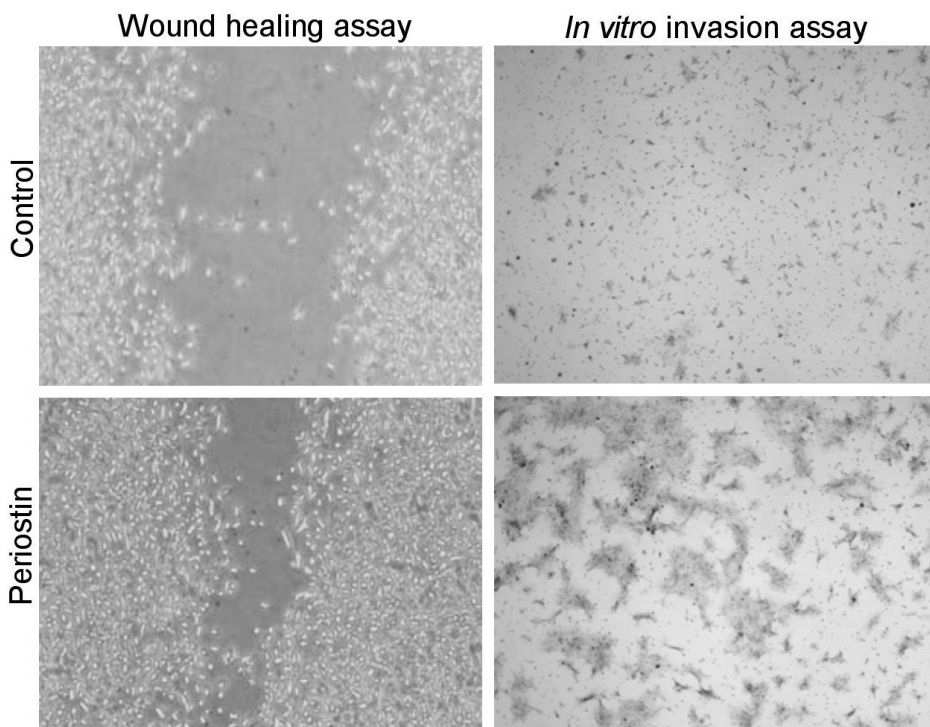

Wound healing assay **In vitro invasion assay**

Control

Periostin

Fig. 3. Periostin overexpressing cells enhanced migration and invasion. Oral cancer cells were engineered to overexpress Periostin by transfection with Periostin plasmid. Migration was assayed by wound healing assay. For the wound healing experiment, cells were seeded on 6 well plates and were allowed to grow to complete confluence. Subsequently, a plastic pipette tip was used to scratch the cell monolayer to create a cleared area, and the wounded cell layer was washed with fresh medium to remove loose cells. Immediately following scratch wounding (0 h) and after incubation of cells at 37°C for 24 h, phase-contrast images of the wound healing process were photographed digitally. The invasiveness of the cells was determined by *in vitro* invasion assay. Invasion was measured by use of a 24 well cell culture insert with 8 mm pores (Falcon, Becton Dickinson, Franklin Lakes, NJ). The filter was coated with 20 μg of EHS extract (Iwaki Garasu, Tokyo, Japan), which was reconstituted basement membrane substance. The lower compartment contained 0.5 ml of medium. After trypsinization, 1.5×10^5 cells were resuspended in 100 μl of medium and placed in the upper compartment of the cell culture insert for 24 hours. To examine the invasiveness, cells were fixed with formalin and stained with hematoxylin. Periostin overexpression enhanced migration and invasion of oral cancer cells.

cancer cells enhanced migration and invasion (Kudo et al., 2006) (Fig. 3). Yan and Shao also found that Periostin overexpressing 293T cells showed increased migration and invasive activity compared to control cells which can be blocked by anti-αvß5 antibody or EGFR kinase inhibitor, tyrphostin-25 (Yan and Shao, 2006). Moreover, Periostin overexpressing 293T cells expressed EMT related genes, vimentin and fibronectin and increased MMP-9 activation (Yan and Shao, 2006). Baril et al. found that Periostin promotes invasiveness by increasing the motility of cells without inducing expression of proteases in pancreas cancer cells (Baril et al., 2006). These results suggest that promoting invasiveness of cancer cells by Periostin may be mediated by integrin or EGF signaling pathway.
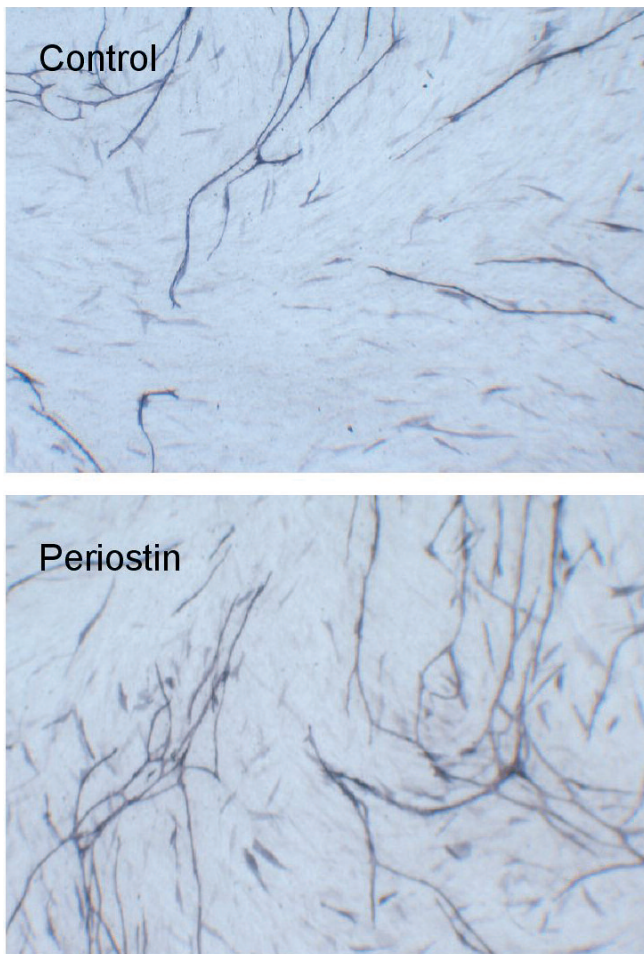

Fig. 4. Periostin enhanced capillary formation *in vitro*. Representative area of capillary formation by recombinant Periostin treatment (0 and 100ng/ml) is shown. An angiogenesis assay kit obtained from Kurabo (Osaka, Japan) was used according to the manufacturer's instructions with minor modification. Human umbilical vein endothelial cells were treated with different concentrations of recombinant Periostin protein (0 and 100ng/ml) and changed media every 3 days. After 12 days, the cells were fixed and stained with anti-human CD31 antibody. Periostin promoted capillary formation.

Cellular survival

Metastatic growth is determined by the balance of cell proliferation and programmed cell death (Hanahan and Weinberg, 2000). The mechanism that promotes cell survival or prevents apoptosis of cancer cells would favor the establishment of metastatic colonies. *In vitro* studies demonstrated that highly vascular metastatic tumors derived from the Periostin producing cells showed fewer apoptotic cells than control cells (Bao et al., 2004). Interestingly, Periostin activated the Akt/PKB pathway via the αvß3 integrin to promote cellular survival in colon cancer (Bao et al., 2004). Tumor cells must overcome cellular stresses such as hypoxia and nutrient deprivation inside the metastatic tumors for successful growth. Periostin seems to promote resistance to serum starvation and hypoxia by cellular survival (Baril et al., 2006). They also demonstrated that Perostin promoted the survival of pancreas cancer cells by inducing Akt phosphorylation through binding to ß4 integrin and activation of PI3 kinase pathway (Baril et al., 2006). Both αvß3 integrins and Akt/PKB pathway have been implicated as playing important roles in promoting cell survival and tumorigenesis (Brooks et al., 1994; Nicholson and Anderson, 2002; Stupack and Cheresh, 2002). Interestingly, Tai et al. reported that treatment with anti-Peirostin antibody activates apoptosis and potentiates the effects of 5-fluorouracil chemotherapy in colon cancer cells (Tai et al., 2005). Thus Periostin plays an important role for survival of cancer cells, and can be a useful option for chemotherapy.

Angiogenesis

Angiogenesis, the formation of new blood vessels, is an important process that occurs during the late stages of tumorigenesis. Angiogenesis involves endothelial cell proliferation, migration, and tube formation and is required for tumor growth. Vascular endothelial growth factor (VEGF) has been demonstrated to play a critical role in the development of tumour vasculature (Folkman, 1996). VEGF and its receptor-2, Flk-1/KDR have been extensively documented to be involved in the induction of angiogenesis during the development of solid tumors (Kim et al., 1993; Millauer et al., 1996; Stacker et al., 2001). VEGF secreted from tumor cells, as well as stromal cells, exerts its angiogenic effects on endothelial cells by the activation of Flk-1/KDR. Integrins play an important role in the activation of endothelial cells as well as tumor cells (Senger et al., 1996; Soldi et al., 1999). Engagement of activated VEGF-2 (Flk-1/KDR) with integrin αvß3 in endothelial cells is required for cell migration and adhesion in response to VEGF (Varner and Cheresh, 1996; Max et al., 1997; Mizejewski, 1999). It has recently been revealed that the presence of Periostin can stimulate metastatic growth by inducing angiogenesis (Bao et al., 2004; Shao et al., 2004). Moreover, Periostin enhances VEGF-C receptor Flk-1/KDR expression in endothelial cells through

integrin αvß3-FAK-mediated signaling pathway (Shao et al., 2004). In fact, recombinant Periostin enhanced capillary formation (Siriwardena et al., 2006) (Fig. 4). Thus, Periostin secreted by tumor cells plays a role in a paracrine manner to augment the survival of endothelial cells and induce neovascularization, an activity consistent with the notion that enhanced survival of endothelial cells within tumors is critical for the successful development of tumor angiogenesis (Brooks et al., 1994; Scatena and Giachelli, 2002; Stupack and Cheresh, 2002). Interestingly, clinical studies of Periostin expression in human cancers have demonstrated that increased expression of Periostin is correlated with the number of blood vessels and metastasis in oral cancer (Siriwardena et al., 2006).

Metastasis

Metastasis is the leading cause of death in cancer patients. Tumor metastatic process consists of multiple and complex steps, all of which must be successfully completed to give rise to the outgrowth of metastatic tumors in a new organ environment (Cavallaro and Christofori, 2000; Chambers et al., 2002; Folkman, 2002). During this process, cancer cells have to overcome many types of stresses such as hemodynamic shearing, loss of adhesion, nutrient depletion, hypoxia, and accumulation of wastes that may all induce cell death. Recent molecular studies have advanced our understanding of the disease and provided a rationale to develop novel strategies for early detection, classification, prevention and treatment. Attempts to identify the genes involved in the metastasis are pivotal for the early prediction of cancer behavior.

Periostin may be a candidate for early prediction of malignant behavior of cancer. In fact, clinico-pathological studies revealed that Periostin overexpression is well correlated with metastasis and poor prognosis in various cancers (Sasaki et al., 2001a,c, 2002, 2003; Bao et al., 2004; Siriwardena et al., 2006). Moreover, *in vivo* studies revealed that Periostin displayed a striking phenotype of greatly accelerated tumour metastatic growth by using the animal model system of metastasis (Bao et al., 2004; Shao et al., 2004; Kudo et al., 2006; Yan and Shao, 2006). This suggests that invasion, cellular survival and angiogenesis mediated by Periostin may be involved in the process of metastasis.

Problems awaiting solution

Cumulating studies indicate that immuno-histochemical expression of Periostin and serum levels of Periostin may have prognostic relevance in cancers. Overexpression or elevated serum levels of this protein appear to be associated with poorer prognosis of various cancers. Why is Periostin overexpressed in cancer? In colon cancer cells, Periostin expression is induced by transforming growth factor ß1 (TGF-ß1) (Tai et al., 2005). Similarly, Periostin is induced by TGF-ß1 in osteoblasts and fibroblasts (Horiuchi et al., 1999). Moreover, other factors such as Twist, IL-4, or IL-13 induced Periostin expression or secretion (Oshima et al., 2002; Takayama et al., 2006). Further studies are required to identify the inducing factor of Periostin in each type of cancer.

On the other hand, Periostin binds to αvß3, αvß5 and α6ß4 integrins, fibronection, tenacin-C, collagen V and Periostin itself (Gillan et al., 2002; Bao et al., 2004; Shao et al., 2004; Baril et al., 2006; Takayama et al., 2006; Yan and Shao, 2006). It is still unclear whether these interactions are involved in tumor progression, including cellular survival, angiogenesis, invasion and metastasis. A mechanistic appreciation for how Periostin mediates cancer cell growth and survivsal, as well as crosstalk with stromal-cell accomplices, will be necessary to identify stages of metastasis that might be susceptible to therapeutic intervention. Importantly, the finding that Periostin influences metastatic potential raises the possibility that it could be used as a molecular target in anti-metastasis therapy of cancer patients.

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