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Review article

The anticancer potential of thrombospondin-1 by inhibiting angiogenesis and stroma reaction during cervical carcinogenesis

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ABSTRACT

Tumor growth is angiogenesis dependent. Angiogenic switch (the acquisition of an angiogenic phenotype) is essential for cervical carcinogenesis. Thrombospondin-1 (TSP-1) is an endogenous angiogenic inhibitor with multiple functional domains and interacting receptors. The disruption of TSP-1 fence (the expression in basal epithelia) occurred concordantly during the transition from low-grade squamous intraepithelial lesion into high-grade squamous intraepithelial lesion. This concordance suggests that TSP-1 plays a role in the regulation of angiogenic switch during cervical carcinogenesis. Tumor vasculature as a therapeutic target offers a paradigm shift for anticancer therapy. Endothelial cells do not appear to acquire resistance during antiangiogenic therapy. Low-and-frequent dose "metronic" chemotherapy is found to be antiangiogenic, which is more effective in targeting tumor endothelia than traditional large, single bolus doses. Meanwhile, the invasion process of cancers is associated with stroma reaction, which is characterized by fibroblasts' activation. In addition to the well-known angiogenesis inhibitor, TSP-1 also has a novel role of blocking activated fibroblasts (myofibroblasts) from invading cancer. Activated fibroblasts during stroma reaction could be used as an efficient drug delivery system to prevent or slow the local growth of cancer cells. Elucidation of the mechanism by which fibroblasts are recruited into cancer stroma could lead to new insights into not only the mechanisms of cancer progression but also strategies for cancer treatment. A better understanding of stromal contributions to cancer progression will likely result in the identification of new therapeutics targeting the stroma.

Angiogenesis and stroma reaction are important during carcinogenesis

Tumor growth is angiogenesis dependent

Angiogenesis is defined as the formation of new blood vessels by proliferation of new capillaries from preexisting microvessels. This process is distinct from vasculogenesis, which is defined as the formation of blood vessel *de novo* from angioblasts.[1,2] Angiogenesis involves degradation of the basement membrane surrounding an existing capillary or venule, migration of endothelial cells through the basement membrane to create a sprout, proliferation of endothelial cells, formation of a lumen within the new sprout and joining of two sprouts to from a functional capillary loop, and vessel maturation.[3,4] The idea that tumor growth is angiogenesis dependent was first proposed in 1971, allowing antiangiogenic therapy to be used to treat cancer.[5] The development of a solid tumor progresses from a prevascular phase to a vascular phase. The prevascular tumor does not induce angiogenesis, is limited in size, and rarely metastasizes. The vascularized tumor induces host microvessels to undergo angiogenesis. The best characterized example is the hypoxia-dependent

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angiogenic switch in which host cell-derived endothelial cells invade into the tumor stroma to form new blood vessels.[6] Thus, blocking angiogenesis can result in tumor dormancy, in which tumors could not expand beyond a microscopic size.[7] Within the dormant tumors, the proliferating tumor cells are balanced by apoptotic tumor cells and few, if any, microvessels.[8]

Angiogenic switch during cervical carcinogenesis

Cervical cancer usually develops by a sequence of gradual, stepwise events starting at low-grade squamous intraepithelial lesion (LSIL) and progressing through high-grade SIL (HSIL), until invasive cancer ensues.[9] Tumor development and metastasis is a complex process that includes transformation, proliferation, neovascularization, and metastatic spread. The angiogenic switch-acquisition of an angiogenic phenotype that is induced by a change in the balance of angiogenesis activators and inhibitors, is essential for tumor growth and metastasis.[2,10] During carcinogenesis, Toussaint-Smith et al[11] and Bequet-Romero and Lopez-Ocejo[12] have described an increase in vascular endothelial growth factor (VEGF) expression associated to the expression of human papillomavirus type 16 oncoproteins E6 and E7. HPV oncoproteins E6 and E7 disrupt the functions of the tumor suppressors p53. Loss of p53 results in upregulation of VEGF and downregulation of thrombospondin-1 (TSP-1). HPV 16 E6 and E7 oncoproteins may contribute to the development of cervical cancer not only by disrupting cell cycle regulation but also by creating a microenvironment that fosters the growth of tumors.[11,12] It has been reported that tumor microvasculature, accompanied by the overexpression of VEGF, was progressively upregulated during the process of cervical carcinogenesis.[13] However, the timing of angiogenic switch during cervical carcinogenesis remains controversial.[14] A debate exists regarding the ability of cervical intraepithelial neoplasia to induce angiogenesis.[14–16] Smith-McCune and Weidner[14] found a significant increase of microvessel density in the cervical intraepithelial neoplasia III lesions compared with those underlying low-grade lesions.[14] By contrast, reports from Abulafia et al[16] show that microinvasive squamous cell carcinoma is angiogenic, but not carcinoma *in situ*. Wu et al[17] examined different severities of cervical lesions in the same slide, to eliminate the heterogeneity. The data showed that the angiogenic switch in cervical carcinogenesis occurred during the transition from LSIL to HSIL, and the neovascularization was largely confined to a narrow zone immediately underneath the dysplastic epithelium. It further suggests that cervical carcinogenesis is angiogenesis-dependent.

Invasion process of cancer cells is associated with stroma reaction

Our understanding of cancer has largely come from the analysis of aberrations within the tumor cell population. There is emerging evidence to highlight the important role of tumor microenvironment in tumorigenesis.[18] Stroma reaction, also known as stromagenesis, is a host response of mesenchymal alteration induced in cancer that produces a progressive and permissive mesenchymal microenvironment, thereby supporting tumor progression.[19] Paget[20] first proposed "seed and soil hypothesis" to highlight the influence of tumor growth by interactions between malignant cells and the tumor stroma in 1889. In the "seed and soil hypothesis" [20] of cancer biology, cancer cells are the "seeds," and the microenvironment is the "soil" in which the "seeds" must find a receptive environment.[21] The normal host microenvironment is nonpermissive for neoplastic progression, and tumor-reactive stroma promotes neoplastic growth and metastasis.[22] Activation of the local invasive environment seems to create a permissive field for the malignant cells.[23]

Stroma reaction is characterized by fibroblast activation

Activated fibroblasts, also called myofibroblasts, are defined by the expression of α-smooth muscle actin, desmin, vimentin, etc. in the fibroblasts.[24] Activated fibroblasts can produce noncellular scaffolds in response to extracellular stimuli, and create an environment promoting tumor progression.[25] In addition, activated fibroblasts within the tumor stroma have a propensity to migrate and invade like cancer cells.[26] The proliferative activity of activated fibroblasts in cancer-induced stroma is closely linked to tumor progression, lymph node, and distant organ metastasis of breast cancer.[27] Normal stromal cells may prevent epithelia from becoming tumorigenic.[28] Fibroblasts, as the major component of stroma, are recruited and can convert into smooth muscle actin-positive fibroblasts, i.e., myofibroblasts or activated fibroblasts, during stroma reaction.[29,30] Myofibroblasts appear at the invasion front during stromal changes in cells.[31]

TSP-1 and cervical carcinogenesis

TSP-1 is a matricellular protein with diverse functions

Thrombospondins (TSPs) consist of a family of five extracellular proteins that participate in cell-to-cell and cell-to-matrix communications.[32] Among them, TSP-1 is a 450-kDa homotrimeric matricellular glycoprotein with potent antiangiogenic effects. In many tumor types, TSP-1 can block *in vivo* neovascularization and decrease malignant tumor growth (e.g., skin, prostate, and bladder cancers),[33,34] whereas in others (e.g., breast cancer) it promotes cancer cell adhesion, migration, and invasion.[35] The differential effects of TSP-1 on tumorigenesis indicate that TSP-1 exerts different biological functions in different cell types. Also, TSP-1 interacts with multiple extracellular macromolecules and cell surface receptors, thus exerting a wide range of responses.[36,37] The hypothesis of this review is "Can TSP-1 could inhibit cancer progression via targeting endothelial cells (EC) in angiogenesis and fibroblasts (F) in stroma reaction?" (Figure 1).

TSP-1 acts as "an angiogenic fence" during cervical carcinogenesis

Wu et al[17] proposed a "TSP-1 fence" in which TSP-1 is mainly localized on basal cervical epithelial cells, and arrayed like a barrier in normal cervical epithelium or LSIL. TSP-1 decreases significantly during the transition from LSIL to HSIL, which is concomitant with the increase of microvessel density counts. The temporal and spatial concordance of TSP-1 downregulation and the emergence of angiogenic imply that the "TSP-1 fence" may act as an angiogenic barrier to inhibit angiogenesis that occurred in the early phase of cervical carcinogenesis. The disappearance of the angiogenic barrier may induce a vigorous angiogenic response for tumor growth and perhaps tumor metastasis.[38] TSP-1 does not appear to contribute directly to the structural integrity of connective tissue elements. Instead, TSP-1 acts by modulating the activity and bioavailability of protease and growth factors and by interaction with cell-surface receptors.[39,40] Matrix metallopreoteases (MMPs) have been shown to play an active role in the neovascularization of tumors through their ability to degrade the extracellular matrix.[41,42] Bergers et al[43] showed that the switch from vascular quiescence to angiogenesis involves MMP-9, which is upregulated in angiogenic islets and tumors. TSP-1 acts as a multifunctional modulator of angiogenesis by modulating through the activity and bioavailability of MMP-9.

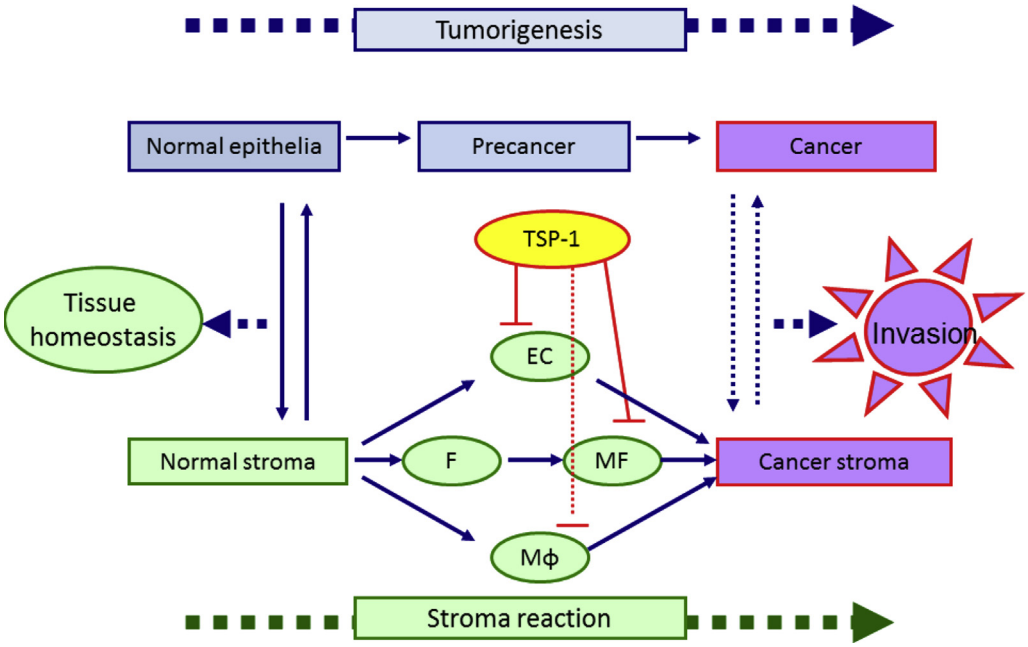

Figure 1. The hypothesis of this review is "Can TSP-1 could inhibit cancer progression via targeting endothelial cells (EC) in angiogenesis and fibroblasts (F) in stroma reaction?". F = fibroblast; MF = myofibroblast; Mφ = macrophages; TSP-1 = thrombospondin-1.

Does TSP-1 play a physiologic gatekeeper role in cervical carcinogenesis?

The loss of the TSP-1 barrier in early cervical cancer lesions leads to more aggressive and more vascular cancer phenotypes.[17] In a related situation, elevated angiogenesis inhibitor endostatin level in conjunction with elevated VEGF are associated with either more aggressive or with metastasis or shortened survival in renal cell cancer, colon cancer, and soft tissue sarcoma.[44–46] These observations showed that the angiogenic inhibitors can be modulated as a result of changes in the tumor environment or in tumor disease burden.[47,48] Although circulating angiogenic activators such as basic fibroblast growth factor, VEGF, and angiogenin have been evaluated not only as diagnostic and/or prognostic factors in cancer patients, little is known about the clinical significance of angiogenic inhibitors. Neither the source nor the mechanism of TSP-1 protein externalization has been clarified in detail.[49] The cause–effect relationship of TSP-1 as a gatekeeper during cervical carcinoma has not been clearly established.[50] The inverse relationship between TSP-1 staining and severity of tumor lesions may be influenced directly or indirectly by other processes (e.g., angiogenic factors). The prevention of blood vessel development appears to be the mechanism of action of many successful chemopreventive drugs of natural or synthetic origin, termed "angioprevention," which hypothesizes that antiangiogenesis is at the basis of tumor prevention, and also suggests that many antiangiogenic drugs could be used for chemoprevention in higher-risk populations or in early intervention.[50] There is a growing body of experimental evidence that antiangiogenic strategies will contribute to the future therapy of cancer.[51] Proof of such a relationship would provide a rationale for the use of angiogenic inhibitors as preventive agents in patients at high risk for developing cancer.

TSP-1 inhibits stroma reaction by inhibiting activated fibroblasts from invading cancer

The downregulation of TSP-1 in cervical epithelium temporally and spatially coincided with the emergence of angiogenic switch during cervical carcinogenesis.[17] However, the exact biologic roles of TSP-1 in tumor stroma reaction and progression remain undetermined. Wu et al[52] demonstrated a temporal inverse correlation of TSP-1 and stromal marker expression during cervical carcinogenesis using human clinical specimens. The inhibitory effect of TSP-1 on stromal marker expression was further confirmed in SCID mouse xenografts using transfection of TSP-1 cDNA expression vectors. Genetic manipulation of TSP-1 expression level in the cells demonstrated that TSP-1-mediated inhibition of stroma reaction was primarily due to the inhibition of activated fibroblast migration and invasion, rather than a direct effect on the stromal maker expression. These results indicate that TSP-1 participates not only in the negative regulation of angiogenesis but also stroma reaction during cervical carcinogenesis.[52] Therefore, TSP-1 has the potential to inhibit tumor progression through blocking the migration and invasion of activated fibroblasts and leading to stroma normalization.[52]

It has become increasingly clear that, from the context of tumor–stroma interactions, stroma plays an active role in tumor progression.[18] Stromal cells can acquire oncogenic transformation following the exposure to carcinogen,[53] manipulation of MMPs,[54] and the recruitment of inflammatory cells to the stroma.[55,56] Stroma reaction is often accompanied with stromal marker expression and functional changes into an invasive phenotype. Inhibition of stroma reaction by TSP-1 might be through reduced expression of stromal markers and invasiveness or inhibition of activated fibroblast migration and recruitment to tumor stroma.

Antiangiogenic therapy offers a paradigm shift for anticancer therapy

Tumor vasculature as a therapeutic target

There exist several limitations in conventional chemotherapy—e.g., it is easy for tumor cells to develop resistance to cytotoxic agents that cause DNA damage or disrupt DNA replication, a phenomenon related to their genomic instability after varying periods of sensitivity.[57] The applied clinical strategy involves

multidrug regimens designed to kill as many tumor cells as possible by administering combined cytotoxic agents at the maximum tolerated dose. The goal is to obtain total eradication of the cancer cells.[58] However, most solid neoplasms are the result of multiple genetic abnormalities and may contain heterogeneous subpopulations of cells with different cell kinetics, and invasive and metastatic properties.[59] High levels of VEGF and low levels of TSP-1 were associated with a shorter survival. These results are in agreement with those observed in a previous study that showed an association between low levels of TSP-1 and high levels of VEGF with a worse survival in nonsmall cell lung cancer. In particular, levels of VEGF and TSP-1 correlated with prognosis and could be useful as prognostic markers.[60] There are some advantages to regard tumor vasculature as the therapeutic targets. Tumor endothelia, as compared with tumor cells, are composed of more genetically stable cells. There is less likelihood of the emergence of acquired chemoresistance. There are fewer systemic side effects and less toxicity. They offer more feasibility of long-term administration and can be combined with other cytostatic and/or molecularly targeted therapy.[58] Systemic administration of inhibitors can easily reach the target at the concentration of drug needed.[58] The proliferation and migration of tumor endothelia can be inhibited by naturally occurring angiogenesis inhibitors (e.g., endostatin, angiostatin, TSP-1).[61,62] In addition, the number of structurally abnormal vessels was reduced, suggesting that these agents may "normalize" the tumor vasculature.[63] Endothelial cells do not appear to acquire resistance to some antiangiogenic agents. It offers the possibility of reinducing a response after interruption of therapy.

Low-dose "metronic" chemotherapy is antiangiogenic

Surprisingly, cytotoxic chemotherapy is found to have antiangiogenic effects, particularly when administered at low and frequent doses. This scheduling is more effective in targeting tumor endothelia than large single bolus doses followed by long rest periods.[62] Conventional cytotoxic chemotherapeutic drugs were designed to treat cancer by directly killing or inhibiting the proliferation of rapidly dividing tumor cells. However, recent studies have highlighted the possibility that cytotoxic agents might reasonably be considered to have meaningful antiangiogenic activity as a secondary mechanism.[64] The use of chronically administered chemotherapeutic agents in frequent, even daily, schedule with no prolonged drug-free breaks at low doses significantly below the maximum tolerated dose, is called "antiangiogenic" or "metronomic" chemotherapy.[65]

The potential advantages of metronomic chemotherapeutic include the following: (1) significantly delay in the onset of mutation-dependent mechanisms of acquired drug resistance, because the target of the therapy is the genetically stable, activated endothelial cells rather than the genetically unstable highly mutable cancer cells[66]; (2) facilitation of the efficacy and durability of long-term integration of chemotherapy drugs with targeted antiangiogenic agents[67]; (3) reduction or loss of traditional toxic side effects due to the high sensitivity and selectivity characters[68,69]; and (4) induction of an antiangiogenic effect by decreasing the mobilization and/or viability of circulating bone marrow-derived endothelial precursor cells.[70]

TSP-1 plays angioinhibitory roles via a mediator of the low-dose metronomic chemotherapy, in addition to direct endothelial targeting. TSP-1 has been shown to possess potent angioinhibitory effects in epithelial tumor development.[34] In addition to the direct targeting effects, Bocci et al[66] reported that protracted exposure of endothelial cells *in vitro* to low concentrations of cytotoxic chemotherapeutic drugs in metronomic low-dose cyclophosphamide, caused marked induction of gene and protein expression of TSP-1. The induction of TSP-1, as a secondary mediator of the antiangiogenic effects, in low-dose metronomic chemotherapy regimens can explain the "indirect" pathway-induced growth arrest or apoptosis of endothelial cells.[71] In summary, TSP-1 may exhibit its antiangiogenic effects in two ways: direct targeting of the endothelium and by acting as a mediator of metronomic chemotherapy.

A novel role for myofibroblasts as a therapeutic target

Host stroma is an active participant during tumor progression, whereas myofibroblasts in the host stroma stimulate cancer invasion. Maintenance of epithelial tissues needs the stroma. When the epithelium changes, then the stroma inevitably follows. The changes in the stroma drive invasion and metastasis, the hallmarks of malignancy.[31] However, Kinzler and Volgestein[72] proposed landscape defect to describe that changes in the epithelial compartment might be secondary to alterations of the stroma reaction that occur prior to epithelial changes. Therefore, the epithelial compartment and stroma compartment might invade each other. The interaction between the epithelial and mesenchymal compartments creates a local heterotypic "invasion field" from which the metastatic cell emerges and disseminates.[73]

Myofibroblasts themselves are invasive, are present in the stroma of many malignant tumors, and are frequently localized at the front of invasion.[31] Myofibroblasts may participate at the transition from the noninvasive toward the invasive phenotype is compatible with their appearance in benign lesions that have a high risk of progression toward invasive cancer. Myofibroblasts invade the tumor site, and this invasion may facilitate angiogenic invasion. During avascular growth of developing hepatic metastases, myofibroblast-like cells are already present, prior to endothelial cell recruitment.[74]

Therapeutic implication via inhibiting angiogenesis and stroma reaction

Tumor angiogenesis can be used as a therapeutic target

Angiogenesis plays a critical role in the growth and metastasis of tumors. The antiangiogenesis effect of metronomic scheduling has caused the paradigm shift from conventional dose-density chemotherapy to metronomic scheduling. Significant antiangiogenic and antitumor effects are unlikely to be achieved in the clinical setting with a single chemotherapeutic agent at metronomic doses. Pioneering studies by Kakeji and Teicher[75] showed the potentiality or synergism when angiogenic inhibitors were combined with standard schedules of certain cytotoxic agents. The efficacy of metronomic chemotherapy can be significantly increased when administered in combination with antiangiogenic drugs, such as antibodies against VEGF or VEGFR2.[67] Browder et al[62] have used cyclophospamide and TNP-470 to reveal the antiangiogenic capability of cancer chemotherapy to eradicate chemoresistant tumors. VEGF promotes angiogenesis, a mediator of disease progression in cervical cancer. Bevacizumab, a humanized anti-VEGF monoclonal antibody, has single-agent activity in previously treated, recurrent disease. Most patients in whom recurrent cervical cancer develops have previously received cisplatin with radiation therapy, which reduces the effectiveness of cisplatin at the time of recurrence. The addition of bevacizumab to combination chemotherapy in patients with recurrent, persistent, or metastatic cervical cancer was associated with an improvement of 3.7 months in median overall survival.[76] The proposed rationale for the beneficial effect of such combinations was based on their ability to

target both the parenchymal and stromal components of neoplasia.[77] Tumor endothelial targeting and tumor cell targeting should not be thought of as mutually exclusive. Antiangiogenic therapy can be added to chemotherapy, radiotherapy, immunotherapy, gene therapy, or any other traditionally cancer cell-directed modality.[78]

As angiogenic inhibitors become more widely used in anticancer therapy, it will be important to reduce the harsh side effects and risk of drug resistance of conventional chemotherapy.[7] The paradigm of anticancer treatment may shift from cancer-centered to epigenic, endothelia-centered therapy.[78] The final goals of anti-angiogenesis therapy are not to cure cancer; instead, it is to make cancer more survivable and controllable, and eventually to be converted to a chronic manageable disease, such as heart disease or diabetes, especially in conjunction with radiation, chemotherapy, and other treatments.[79]

Stroma reaction can be used as a therapeutic target

In viewing the fact that stromal therapy has recently emerged as a strategy for cancer treatment, the clinical studies have shown the effective treatment for many types of cancer.[80] TSP-1 is potentially an example of a molecule that is capable of altering the composition of the tumor stroma, in addition to inhibiting angiogenesis. By inhibiting vascular cells, inhibiting recruitment, and preventing fibroblast activation, TSP-1 may play a role in “normalizing” the tumor stroma and creating a microenvironment that is on-permissive for tumor growth.[52] Myofibroblasts, the major component of stroma, play a major role in tumor progression. However, the definite role of TSP-1 in myofibroblasts remains to be determined. Both the upregulation[81] and repression[82] of TSP-1 have been reported to cause NIH3T3 in the malignant phenotype. It has been reported that TSP-1, in cooperation with integrin, affects focal adhesion kinase-dependent signaling to induce focal adhesion disassembly and spreading.[83,84] Further work on the biologic mechanisms of myofibroblast recruitment is needed, before myofibroblasts can be used as a therapeutic target or biologic tracer of cancer cells.

Conclusion

After the clinical observation that TSP-1 plays an important role in the regulation of angiogenic switch during cervical carcinogenesis, Wu et al[17] further elucidated the cause–effect relationship between TSP-1 and tumor–stroma interaction.[52] TSP-1 reduces the stroma reaction by changing the behaviors of myofibroblasts. Although further work on the biologic mechanisms of fibroblast recruitment is needed, the present study may offer the evidence that TSP-1 can change the tumor–stroma reaction during tumor progression by acting on the activity of myofibroblasts.[52] It suggests that fibroblasts can be used as a biologic tracer of cancer cells and could act as an efficient drug delivery system to prevent or slow the local growth of cancer cells. Elucidation of the mechanism by which fibroblasts are recruited into cancer stroma could lead to new insights into not only the mechanisms of cancer progression but also strategies for cancer treatment. A better understanding of stromal contributions to cancer progression will likely increase our awareness of the importance of the combinatorial signals that support and promote growth, dedifferentiation, invasion, and ectopic survival and eventually result in the identification of new therapeutics targeting the stroma.[31]

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