The roles of thrombospondin-1 in tumor angiogenesis and stroma reaction during cervical carcinogenesis

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Abstract

The acquisition of an angiogenic phenotype (angiogenic switch) is essential for cervical carcinogenesis. The angiogenic switch is balanced by the angiogenic activators and inhibitors. Thrombospondin-1 (TSP-1) is an endogenous angiogenic inhibitor with multiple functional domains and interacting receptors. Our study was firstly aimed to examine the spatial and temporal relationship of TSP-1 expression in patients with squamous cell carcinoma of uterine cervix and its precursor lesions, and to correlate its expression with tumor angiogenesis. Our results indicate the disruption of TSP-1 fence (the expression of TSP-1 in basal epithelia) and the switch to angiogenic phenotype occurred concordantly during the transition from low grade squamous intraepithelial lesion (LSIL) into high grade squamous intraepithelial lesion (HSIL). This concordance suggests that TSP-1 play a role in the regulation of angiogenic switch during cervical carcinogenesis. We conclude that the onset of angiogenesis is an early event in cervical carcinogenesis due, in part, to the down-regulation of TSP-1 by the dysplastic epithelium.

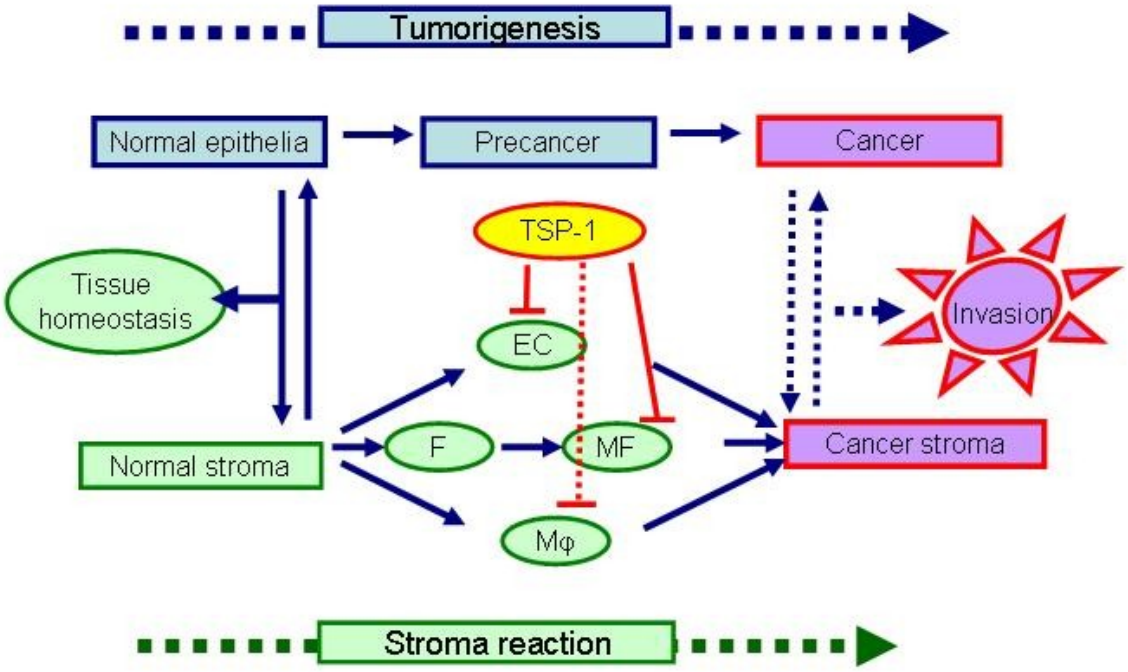
Stroma reaction (also called stromagenesis) is a host reaction of stroma cells that, when induced in cancer, produces a progressive and permissive mesenchymal microenvironment, thereby supporting tumor progress. In addition to the well-known angiogenesis inhibitor, TSP-1 has been shown to exert different biological functions on various stromal cell types, e.g. fibroblasts. Therefore, we hypothesized that TSP-1 may play a role in stroma reaction, characterized by fibroblast activation. We firstly tried to elucidate the correlation between the TSP-1 expression and the overexpression of stroma markers in human cervical lesions; secondly, we tried to elucidate whether TSP-1 can exhibit its anti-tumor effects through the angio-inhibitory effects and the ability to

inhibit tumor stroma reaction in SCID mice xenotransplant model; thirdly, we tried to elucidate whether TSP-1 can change the stroma reaction by inhibiting the migration and invasive ability of myofibroblast (activated fibroblasts) from invading tumor cell cluster. Our results revealed: First, immunohistochemistry staining of human clinical specimens showed the disappearance of TSP-1 coincided with the emergence of the overexpression of two stromal markers, α-SMA and desmin, in a stepwise pattern. Second, transfection of SiHa cervical cancer cells with a plasmid expressing the TSP-1 protein exhibited anti-angiogenic activity in vitro, and resulted in reduced tumor growth in SCID mice, which was accompanied by a decrease in tumor vascularization and lower expressions of α-SMA and desmin than those in the vector controls. Third, transfection with TSP-1 and purified TSP-1 added to NIH3T3 cells did not alter the protein levels of α-SMA and desmin which was increased by transforming growth factor β (TGF-β), a potent fibroblast activation and transdifferentiation factor, but significantly inhibited matrix metalloprotease-2 (MMP-2) activity. The increased migration ability and the invasive ability into tumor cluster of TGF-β-treated-NIH3T3 cells were dose-dependently inhibited by TSP-1. In contrast, ectopic TSP-1 expression in SiHa cells has little effect on the invasive ability of the NIH3T3 cells.

Together, these data demonstrate that TSP-1 possesses a novel role to reduce the expression of stromal markers in both human clinical specimens, and an *in vivo* tumor model. The inhibitory ability of TSP-1 to reverse stroma reaction could be partly attributed to the blockage of myofibroblasts from invading cancer. By targeting the tumor-stroma microenvironment, treatment effectiveness could be increased

Keywords: angiogenesis, α-smooth muscle actin, desmin, cervical neoplasms,

(myo)fibroblasts, matrigel, stromal reaction, thrombospondin-1



Summary TSP-1 inhibited cancer progression via inhibiting stroma reaction. Stroma reaction occurs parallel to tumorigenesis. In addition to the epithelial component, host stroma, including endothelial cells, (myo)fibroblasts, and inflammatory cells, e.g. macrophage, etc, plays an active participant. TSP-1 can inhibit angiogenesis status, the migration and invasive ability of myofibroblasts. (F: fibroblast; MF: myofibroblast; Mφ: macrophages).