Tumor microenvironment research in China

Caihong Zhang\textsuperscript{a} and Qiangze Zhang\textsuperscript{a,b}

\textsuperscript{a}Life Science Laboratory, Nankai University, Tianjin, China
\textsuperscript{b}College of Pharmacy, State Key Laboratory of Medicinal Chemical Biology and Tianjin Key Laboratory of Molecular Drug Research, Nankai University, Tianjin, China

\begin{abstract}
Tumor microenvironment plays a key role in regulating tumorigenesis, progression, and metastasis of cancer. Although it is well investigated the interactions between cancer cells and tumor microenvironment, many mechanisms still retain unknown. In the last several years, Chinese scientist achieve many accomplishment in tumor microenvironment research field refer to tumor immune escaper and anti-tumor immunity; angiogenesis, lymphangiogenesis and tumor therapy; epigenetics and tumorigenesis; proliferation, angiogenesis, invasion and metastasis of cancer; cancer cell, stromal cells and their chemokines or cytokines; the molecular mechanisms of cancer invasion and metastasis. Especially, the molecular mechanism has been well elucidated in epigenetics of breast cancer and anti-angiogenesis, anti-lymphangiogenesis by Endostatin and VEGI (TNFSF15).

\textbf{Keywords}: tumor microenvironment, anti-tumor immunity, anti-angiogenesis, anti-lymphangiogenesis.
\end{abstract}

\section*{INTRODUCTION}

Currently, The Chinese government has invested much money in the cancer research field, especially, in cancer microenvironment research, because of more and more cancer patients in China. Chinese scientists have made a lot of effort to illuminate the reason of tumorigenesis, progression, and metastasis, and sought prevention and treatment for cancer. This review dedicates to introduce the tumor microenvironment research of Chinese scientist in the last decade. These researches of tumor microenvironment include that 1) tumor immune escaper and anti-tumor immunity, 2) angiogenesis, lymphangiogenesis and tumor therapy, 3) epigenetics and cancer, 4) cancer cell and stromal cells, 5) cancer invasion and metastasis.

It is worth mentioned that epigenetics of breast cancer and anti-angiogenesis and anti-lymphangiogenesis by Endostatin and VEGI had attracted the close attention of the international counterparts. Shang’s lab coming from Peiking University illuminates the epigenetics mechanism of increased incidence of endometrial cancer\cite{1} by tamoxifen, and LSD1 mediating breast cancer metastasis\cite{2} on Nature and Cell journal. Luo’s lab coming from Tsinghua University develops an anti-tumor drug, Endostar, has been formally approved Chinese Food and Drug Administration. Another lab coming from Nankai University, professor Li lu-yuan systematically elucidates the mechanism of VEGI anti-tumor, and the recombinant protein VEGI is preparing to clinical trials.

\textbf{Tumor immune escaper and anti-tumor immunity}

One hallmark of cancer cells is immune escape\cite{3}. It is generally known that CD11b+ Gr-1+ myeloid cells (myeloid-derived suppressor cells, MDSC) relating to escape anti-tumor immune can inhibit T cells and dendritic cells. Interestingly, MDSC increase markedly in liver can down-regulate the NK cells function, and inhibit cytotoxicity, NKG2D expression, and INF-\(\gamma\) production of NK cells\cite{4}. When adding TGF-\(\beta\) neutralization antibody into the coculture system of MDSC or NK cells could restore NKG2D expression, cytotoxicity, and INF-\(\gamma\) production of NK cells, whereas supplying exogenous recombinant TGF-\(\beta\) could not significantly suppress NKG2D expression, cytotoxicity, and INF-\(\gamma\) production of NK cells\cite{4}. Furthermore, MDSC, but not Tregs, can significantly restored...
Specific antitumor immunity is a promising approach for cancer therapy. Exosomes derived from heat-stressed tumor cells (HS-TEX) are a population of nanometer-sized membrane vesicles that can induce specific antitumor immunity[8]. HS-TEX contain more HSP60, HSP90, and chemokines compared to those exosomes derived from the untreated-tumor cells (Exo)[9]. Releasing chemokines from exosomes via lipid raft-dependent pathway can chemoattract and activate dendritic cells (DC) and T cells. CD8+ T cells are the predominant T cell subset responsible for the antitumor effect of HS-TEX. In addition, HS-TEX can more efficiently induce maturation of dendritic cells in phenotype and function. CEA+/HS-TEX has superior immunogenicity than CEA+/Exo in inducing CEA-specific immunity[8].

Angiogenesis, lymphangiogenesis and tumor therapy

He’s lab construct transgenic mouse targeting the VEGFR3 coding region for the ligand-binding domain (Vegfr3ΔLBD) or the tyrosine kinase domain with an inactivation point mutation (Vegfr3TKmut) to investigate angiogenesis and lymphangiogenesis. They found that VEGF-C&D/VEGFR3 signal and kinase activity are required for lymphangiogenesis but not for angiogenesis[17]. Zhu et al investigated AKT knockout mouse discovered that loss of Akt1 led to abnormal lymphatic development with reduced lymphatic endothelial cell number and vessel size, smooth muscle cell coverage, and defective valve development compare to wildtype, but lymphangiogenesis was induced by restoring VEGF-C expression in lacking Akt1 of adult mice [18]. Phosphorylated Akt is a central enzyme known to promote VEGF-induced endothelial NOS (eNOS) activation. PKA, another protein kinase, can also activate eNOS. Endothelium-specific Gab1 KO mice showed that their vessels and ECs were defective in vascular sprouting and tube formation induced by VEGF. Furthermore, the phosphorylation of Akt was increased whereas PKA activity was significantly decreased in VEGF-induced the vessels and ECs. Reexpressing active forms of PKA could rescue VEGF-induced eNOS activation and tube formation in EGKO ECs. These results suggested that Gab1 regulates VEGF-induced angiogenesis by the PKA-eNOS pathway[19]. Yet, TNF/TNFR2 signal recruiting lot of macrophages infiltration was nitric oxide dependent to prevent tumor growth by efficiently inhibited angiogenesis[20].

Vascular endothelial cell growth inhibitor (VEGI; TNFSF15), a tumor necrosis factor (TNF) family member[21, 22], is produced by endothelial cells[22], induces apoptosis in proliferating endothelial cells[23], and is down-regulated in tumor vasculature[22]. VEGI is a critical component of the negative control mechanism to tumor angiogenesis in the cancer microenvironment. VEGF and MCP-1 derived from cancer cells, tumor-infiltrating macrophages, and Treg cells effectively inhibit VEGI production from endothelial cells to promote neovascularization in ovarian cancer[24]. Li’s lab found that VEGI can inhibit bone marrow derived Sca1+ hematopoietic stem cell differentiation into endothelial progenitor cells (EPC)[25]. VEGI-induced apoptosis of differentiated EPC is, at least partly, mediated by death receptor-3 (DR3) [25]. It was known that bone marrow derived endothelial progenitor cells contribute to tumor neovascularization. Furthermore, VEGI can inhibit bone marrow-derived endothelial progenitor cell incorporation into tumor vascular to prevent the growth of Lewis lung carcinoma tumors[26]. Endostatin, a 20 kD proteolytic fragment of collagen XVIII, is an endogenous angiogenesis inhibitor and has potent anti-endothelial and
anti-lymphangiogenesis functions[27, 28]. Nucleolin, an endostatin receptor, expressed on the cell surface of vascular and lymphangiogenic endothelial cells, can mediate antiangiogenic and antitumor activity, and dramatically reduce tumor-associated lymphangiogenesis and lymphatic metastasis by combining with endostatin[27, 28]. Chen et al found that phosphatidylinositol 4-kinase type IIa (PI4KIIa) may regulate HIF-1a through the HER-2/PI3K, ERK cascade to promote tumor growth[29]. Knockdown of PI4KIIa eliminates tumor-induced angiogenesis, endothelial cell migration, tube formation and the expression level of VEGF[29]. Together, anti-angiogenesis and anti-lymphangiogenesis, for an example to target vascular and lymphatic endothelial cells, are important fields in cancer therapy.

Epigenetics and cancer
Epigenetics dysregulation is a significant reason of carcinogenesis and Metastasis. Shang et al found that ZIP, a novel zinc finger and G-patch domain-containing protein, can recruit the nucleosome remodelling and deacetylase(NuRD) complex which acts as a transcription represor for EGFR to inhibit cell proliferation and suppress breast carcinogenesis, once ZIP depletion leads to a drastic breast cancer growth in vivo[30]. Lysine-specific demethylase 1 (LSD1), an integral component of the Mi-2/NuRD complex, interacts directly with MTA proteins to inhibit the invasion and metastatic potential of breast cancer cells[2]. The demethylase, JARID1B, specifically demethylated tri- and di-methylated forms of histone H3 lysine 4 (H3K4), and combining with LSD1/NuRD to form the JARID1B/LSD1/NuRD complex can decrease the expression of CCL14 to suppress the angiogenesis and metastasis in breast cancer[31]. JMD2B, a H3K9 trimethyl demethylase, can coordinate H3K4/H3K9 methylation and promote breast carcinogenesis through estrogen receptor α (ERα) pathway, but H3K9 demethylation is a prerequisite for H3K4 methylation[32]. PAX2, as a downstream target of ERα, is crucially to promote cell proliferation and tumorigenesis in the endometrium[1]. Estrogen and tamoxifen can activate PAX2 expression by hypomethylising its promoter in endometrial carcinomas but not in normal endometrium. SET8-directed H4K20me1 is a dual epigenetic mark on the E-cadherin and N-cadherin promoters, and cooperating with TWIST to promote EMT and invasion of breast cancer cells via decreasing the expression of E-cadherin and increasing the expression of N-cadherin[33]. In all, epigenetics widely take part in tumorigenesis, proliferation, angiogenesis, invasion, and metastasis of cancer.

Cancer cell and stromal cells
The stromal cells in tumor microenvironment were composed of cancer-associated fibroblast (CAF), endothelial cell (EC), pericytes (PC), cancer stem cell (CSC), cancer cell (CC), invasive cancer cell, immune inflammatory cells (ICs), and local and bone marrow derived stromal stem cell or progenetor cells[3]. Wang and their colleagues found that CAFs overexpressing PGK1 can increase the invasion of prostate cancer cells via expressing MMP-2, 3 and activating the AKT and ERK pathways[34]. Stromal fibroblasts and tumor cells were coinject into mouse can promote tumor growth, but IFN-γ can down-regulate the VEGF production of fibroblasts to block tumor growth by inhibiting angiogenesis[35]. Tumorigenesis is often accompany with higher fibroblast proliferation, extensive fibrosis, and chronic inflammation. Carcinogen, TPA, can proliferate and accumulate massive FSP1+ fibroblasts to induce skin carcinogenesis through maintaining MCP-1-recruited macrophage infiltration and chronic inflammation[36]. DMBA/TPA-induced IFNγ can up-regulated TNFα, IL-6, TGFβ, IL-17 to promote tumorigenesis by enhancing Th17-associated inflammation[37]. However, IL-15 overexpression in antigen-specific CD4+ T cells can enhance themselves proliferator and anti-tumor effect[38]. Qin et al found that T and B lymphocytes producing IFN-γ is not necessary for tumor immunity, but IFN-γ from innate immune cells (such as NK1.1+ cells and CD11b+ cells) is sufficient for tumor immunity[39]. Furthermore, IL-2 can mediate the cooperation between T cells and innate immune cells to regulate IFN-γ-induced tumor rejection[39]. The chemokine CXCL16 and its receptor CXCR6 regulate the expression of the proangiogenic factors IL-8 or VEGF to participate in the regulation of tumor angiogenesis by the CXCR6/ACT/mTOR pathway[40]. Stromal-derived factor-1α (SDF-1α) was induced in endothelial cells by PDGF-BB derived from tumor through PI3K/Akt/mTOR pathway, and can increase the motility of pericytes and recruit them to angiogenesis[41]. So, chemikines and cytokines also play significant roles in tumorigenesis and cancer growth. The relation of stromal cells and tumor was regard as that of soil and seed.

Cancer invasion and metastasis
Metastasis and invasion are the leading cause of death in cancer patients and the important fields of tumor study. Cellular prion protein (PrPc) is a glycosylphosphatidylinositol (GPI) -anchored membrane protein and play a pole in cell adhesive and membrane signaling. Recently, Fan et al found that PrPc was higher expressed in metastatic gastric cancers than nonmetastatic ones[42]. PrPc increasing MMP11 expression via activating phosphorylated Erk1/2 can significantly promote the adhesive, invasive, and metastatic abilities of gastric cancer[42]. PrPc can also enhance the tumorigenesis and proliferation of gastric cancers by PI3K/Akt pathway and regulate the G1/S phase transition throught activating CyclinD1 signal[43]. Runx3, a RUNX family member, can up regulate the expression of TIMP-1 by two RUNX3 binding sites in the TIMP-1 promoter and suppresses gastric cancer metastasis because of inactivating MMP9 [44]. Luo and his colleagues found that primary tumor can up-regulate angiopoietin 2 (Angpt2), MMP3, and MMP10 to disrupt the vascular integrity in lung which enhanced permeability of pulmonary vasculatures for preparing
Epithelial–mesenchymal transition (EMT) is an important process that promotes cancer invasion and metastasis. EMT has several functional markers including increased the abilities of migration, invasion, scattering, and elongation of cell shape, and accompany with increasing the expression of N-cadherin, Snail, Twist, MMP, and decreasing the expression of E-cadherin[56]. A key regulator in cytoskeleton formation, RhoE, can increase cell invasion and EMT under hypoxic conditions by up-regulating the mesenchymal marker vimentin and MMP2/MMP9, and down-regulating the epithelial marker E-cadherin[57]. An HBPx-upregulated gene, URG11, can also induce EMT under hypoxia environment by activating the β-catenin/TCF pathway and suppressing the expression of E-cadherin[58]. So EMT is a significant transformation to increase tumor metastasis under hypoxia conditions.

**Prospect**

Although scientists have resolved many mechanisms of tumorigenesis, progression, and metastasis of cancer, the new problems are re-emerging with in-depth understanding of the tumor microenvironment complexity. Metastasis is the leading cause of death in cancer patients. How to strictly define the niche of cancer stem cell? Whether and how can CSC niche regulate the cancer metastasis to distant target organs? Whether is the CSC niche similar or equal with adult stem cell niche in body organs that were metastasized usually by specific cancer cells? How to repress the cancer growth by anti-immunity and prevent the tumorigenesis? Many problems still present in cancer research and need to be resolved in the future because of more and more cancer patients.

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**REFERENCES**


