DOCTORAL THESIS

Roles of stanniocalcin-1 on tumorigenicity of hepatocellular carcinoma and regulation of macrophage functions

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Date of Award:
2020

Abstract

The glycoprotein stanniocalcin-1 (STC1) is a paracrine factor in mammals which plays roles in various (patho)physiological functions, such as inflammation and carcinogenesis. Considerable numbers of studies showed dysregulation of STC1 expression in different types of human cancers. A previous study from our group, using clinicopathological data of 216 hepatocellular carcinoma (HCC) patients revealed greater STC1 gene expression in tumors than the paired normal samples. However, patient samples with greater STC1 level exhibited smaller tumor size. In fact, multiple cell types, growth factors and matrix components in tumor microenvironment (TME) control cancer progression. Emerging evidence support the important role of infiltrating immune cells on tumor progression. Among those, tumor associated macrophages (TAM) in TME is known to be an essential driver of tumor inflammation and progression, exerting a yin-yang influence to determine if the tumor is suppressed or paving the way to metastasize. Hepatocellular carcinoma (HCC) is mainly caused by chronic inflammation. With hindsight, the roles of STC1 in inflammation and carcinogenesis were documented. However, the observation on the negative correlation of STC1 expression with tumor size in HCC patients and the roles of STC1 on the interactions between tumor cells and macrophages are not clear.

In Chapter 2, the inverse correlation of STC1 expression with tumor size was addressed. Human metastatic HCC cell line, MHCC97L which was stably transfected with empty vector (P) and STC1 (S1) were used. Nude mice xenograft model showed that tumor size and volume formed from S1 cells were significantly smaller than that from P cells. The observation agreed with the clinical data aforementioned. *In vitro* studies demonstrated S1 cells had lower plating efficiency, migratory and proliferative potential, illustrating a lower tumorigenicity. Biochemical analyses on the rate of glycolysis, extracellular O_2 consumption, ATP production and Western blot studies on mTOR/p70S6K/rpS6 pathway showed the S1 cells adopted a lower energy metabolism. The data may explain the negative correlation between STC expression level and tumor size.

In cancer microenvironment, infiltration of host immune cells, especially macrophages, contributes to inflammation and tumor progression. In Chapter 3, it was hypothesized that cancer cell-derived STC1 alter macrophage functions. Therefore, the effects of STC1-overexpressing MHCC97L on macrophages were

studied. To mimic their interactions, Boyden chamber insert model was adopted to co-culture MHCC97L (97L/P and 97L/S1) and THP-1. Our data illustrated 97L/S1 suppressed migratory response of THP-1, with or without the addition of monocyte chemoattractant protein-1 (MCP-1) as the chemoattractant. Quantitative PCR showed downregulation of cytokine/chemokine receptors (CCR2, CCR4, CSF-1R) in THP-1 when co-cultured with 97L/S1. This prompted us to study the alterations of pathways related to cell motility in THP-1 by 97L/S1. Transcriptomic analysis detected 1784 differentially expressed genes (DEGs) between THP-1 cells co-cultured with 97L/P and 97L/S1. Ingenuity Pathway Analysis (IPA) prioritized an inhibition of RhoA signaling, which is known to stimulate cell motility. Western blotting analysis supported the IPA prediction and the cell migration data to show a significant reduction of MLC2 phosphorylation, leading to impaired formation of stress fibers, cell contraction and cell motility.

The preceding chapters focused on cancer cell-derived STC1 on HCC cells or THP-1 derived macrophages. In Chapter 4, it was hypothesized that macrophage-derived STC1 may also play a role in macrophage differentiation and inflammation, which modulate tumorigenicity of HCC during macrophage-cancer cell interactions. Thus, the roles of endogenous STC1 in macrophage differentiation and functions were investigated. Using human leukemia monocytic cell line THP-1, a pilot study showed a treatment with phorbol 12-myristate 13-acetate (PMA) significantly upregulated STC1 expression and pro-inflammatory cytokines. In follow-up studies, THP-1 was pharmacologically stimulated to differentiate into (i) classically activated macrophages (CAM)/ M1 state, and (ii) alternatively activated macrophages (AAM)/ M2 state. Greater STC1 expression was found to be associated with CAM. To examine the role of STC1 in CAM, $siRNA_{STC1}$ was used for gene knockdown. Conditioned medium collected from $siRNA_{STC1}$-treated CAM inhibited migration of HCC cell line Hep3B. Transcriptomic analysis of $siRNA_{STC1}$-treated CAM revealed an upregulation on TBC1D3G gene, which is involved in the release of extracellular vesicles (EVs) in macrophage to mediate inflammation. This study demonstrated the association between STC1 and macrophage-mediated inflammation.

Collectively, the above studies elucidated the influence of STC1 on cancer cell metabolism, macrophage differentiation and function. It warrants further investigations to unravel the therapeutic potential of STC1 in inflammation and carcinogenesis.

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