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Role of the tumor microenvironment in driving intratumoral spatial niche formation in clear cell renal cell carcinoma

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ccRCC is characterized by considerable intratumoral genetic heterogeneity as well as phenotypic and functional heterogeneity. Several studies have suggested that distinct environmental features such as oxygen and the availability of nutrients, acidity, and proximity to inflammation or immune cells within a tumor could form distinct niches to generate a spatial organization of tumor. However, how cancer cells with different phenotypes distribute spatially within the tumor and what the underlying molecular mechanisms for the formation of distinct niches in the tumor are, has yet to be revealed. Our previous study found that ccRCC cells located at the tumor periphery exhibited an enhanced proliferation rate and an elevated mTOR signaling activity when compared to the tumor center. This implies the existence of intratumoral niches with a spatial organization into tumor periphery and center.

Herein, this study not only confirms the tumor center/periphery model of ccRCC by showing their functional and phenotypic heterogeneity, but also provides a model to explain the potential mechanisms underlying the tumor periphery/center niche organization. The results showed that cancer cells in the tumor periphery were more proliferative as indicated by nuclear Ki-67 staining and cytoplasmic $p27^{Kip1}$ staining than cells in the tumor center. To elaborate these observations, CM was made by culturing the normal peritumoral kidney tissue. Further molecular investigation using a cell culture system revealed that CM were able to drive the proliferation of cancer cell by downregulating $p27^{Kip1}$. Next, a neutralization assay was performed to identify the specific growth factors in the CM for such an effect. It was found that bFGF in the CM was responsible for the more proliferative phenotype of the cancer cells. The

immunoblot and flow cytometry experiments based on the cell culture system revealed that bFGF was able to drive RCC cells to proliferate by activating the PI3K/AKT pathway via Src and then downregulating $p27^{Kip1}$ via the Emi1/Skp2/$p27^{Kip1}$ axis. In addition, immunofluorescence staining showed that PI3K/AKT inhibition led to an accumulation of nuclear $p27^{Kip1}$ in cancer cells, indicating that bFGF may drive a cytoplasmic accumulation of $p27^{Kip1}$ via activation of the PI3K/AKT pathway. Lastly, the prognostic role of the activated form of Src, p-Src Y419, in a TMA cohort consisting of 449 patients was investigated using IHC staining. Both the Kaplan-Meier analysis and the multivariate Cox regression analysis indicated that a high level of cytoplasmic p-Src Y419 was a negative prognostic marker for ccRCC patients.

In summary, this study suggests that the tumor periphery and center are different niches, which harbor functionally different tumor cells, with those at the periphery being more proliferative. bFGF originating from the normal peritumoral kidney tissue plays a critical role in driving this spatial niche formation. This tumor-microenvironment interaction mediated by bFGF also involved the PI3K/AKT and Src pathways, and the Emi1/Skp2/$p27^{Kip1}$ axis. These molecular networks provide potential therapeutic targets for the ccRCC patients. Moreover, a high level of Src activation predicts an adverse prognosis of patients with ccRCC, which further highlights the role of the pathway in ccRCC progression.