PLENARY LECTURES

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CELLULAR PLASTICITY AS A DRIVING FORCE IN CANCER PROGRESSION: THE REGULATORY ROLE OF ADAPTOR PROTEIN RuK/CIN85

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Introduction. Tumor progression is a complex process consisting of several stages including initiation, primary tumor growth, invasion, dissemination and metastasis, which differ by gene expression patterns, level of cell differentiation, proliferation rate, cell motility etc. Metastatic process requires epithelial cells to lose their polarity and intercellular junctions, but to acquire mesenchymal properties, such as cytoskeleton reorganization and increased motility. Such reversible process is called EMT (epithelial-to-mesenchymal transition). It enhances tumor cells motility and dissemination, and also allows cancer cells to acquire stem-like properties and chemoresistance. EMT in cancer cells is triggered through up-regulation of EMT-related transcription factors (TFs), called also EMT master regulators, in response to hypoxia, TGFβ, Notch, Wnt signaling. These TFs, including Snail1, Slug, Twist, ZEB1/2, induce expression of mesenchymal markers (N-cadherin, vimentin, fibronectin) and repress expression of epithelial markers (E-cadherin, claudin, occludin). Study of circulating tumor cells (CTCs) demonstrated several modes of cancer cell migration and metastasis. Mesenchymal type of migration implies spindle-like shape of cancer cells, as well as decomposition of extracellular matrix through increased expression levels and activity of extracellular proteases (MMPs, catepsins, serine proteases). In contrast, amoeboid migration of tumor cells is characterized by rounded shape of migrating cells, cortical actin ring and bleb-like membrane protrusions formation, independency of pericellular proteolysis and increased RhoA-ROCK-dependent signaling. This morphofunctional interconversion is maintained at balanced equilibrium that may be defined as reversal epithelial-mesenchymal-amoeboid transitions. EMT process in tumor cells is strongly linked to dedifferentiation and acquisition of stem-like phenotype. Cancer stem cells (CSCs) are known as small self-renewing subpopulation of tumor cells enable to initiate tumor growth. They are characterized by activation of embryonic signaling pathways (Wnt, Notch, Hedgehog), expression of specific markers (e.g. markers of breast CSCs: $CD44^{+}/CD24^{low/-}$, CD133, ALDH) and increased radio- and chemoresistance. Epithelial-mesenchymal plasticity includes profound changes in cellular signaling. So, identification and targeting the signaling regulators is of high importance strategy to combat the spread of cancer cells. One of such potential regulators is adaptor protein Ruk/CIN85, consisting of three SH3 domains, proline-rich motifs, and coiled-coil domain. It was described as a component of EGFR (and other RTKs) endocytosis complex, and also was found in multimolecular complexes regulating cell proliferation, motility, adhesion and survival. High amounts of this adaptor were found in tumors of different tissue origins and metastatic loci. All these features determine the need of further investigations on the role of Ruk/CIN85 in the control of epithelial-mesenchymal plasticity. Thus, the main aim of present study was to elucidate the role of Ruk/CIN85 in acquisition and maintenance of cancer cells plasticity.

Methods. In order to study the role of adaptor protein Ruk/CIN85 in the control of cancer cells plasticity we used mouse breast adenocarcinoma 4T1

cells with different levels of Ruk/CIN85 expression. Cancer cells invasiveness was studied using Boyden chamber assay, metastatic potential was estimated by experimental and spontaneous metastasis *in vivo* models. The expression levels and/or content of specific EMT, CSCs and reprogramming markers were evaluated by RT-qPCR, Western-blotting and/or immunofluorescent microscopy. Statistical analysis was carried out using ANOVA with Newman-Keuls correction.

Results. Our study demonstrated increased invasiveness and metastatic potential of Ruk/CIN85-overexpressing 4T1 cells and suppression of these abilities in 4T1 cells with Ruk/CIN85 knockdown. Analysis of EMT, CSCs and reprogramming markers showed mixed mesenchymal-amoeboid state with pronounced stem cells features in 4T1 cells with Ruk/CIN85 up-regulation, while Ruk/CIN85-downregulated cells became more epithelial. The obtained results allow us to suggest that high levels of Ruk/CIN85 are associated with cancer cells plasticity and, consequently, increased invasiveness and metastasis, while low levels of Ruk/CIN85 lock tumor cells in the rigid epithelial state resulting in the loss of plasticity.

Conclusions. Cancer plasticity phenomenon combines such features of tumor cell as EMT, stemness and reprogramming, allowing tumor cells adapt to the microenvironment and successfully metastasize. It this study we revealed the role of adaptor protein Ruk/CIN85 as a key regulator of epithelial-mesenchymal plasticity in 4T1 breast cancer cells.

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