Poznań, 11 of September 2017

Thesis dissertation review

by Mohammed Imran Khan

„Comparative Gene Expression profiling of Primary and Metastatic Renal Cell Carcinoma Stem Cell-Like Cancer Cells"

Renal cancer accounts for app. 2-3% of all malignant cancer types. There are about 270,000 cases diagnosed worldwide and 116,000 deaths caused by renal cell carcinoma (in Poland 4700 new cases and 2500 deaths). Renal cell carcinoma (RCC) is the most common histology found in kidney tumors, with clear cell RCC being the most histology subtype (70-80% of all renal malignancies). Metastatic RCC is the spread of the primary renal cell carcinoma from the kidney to other organs. 25-30% of people have this spread by the time they are diagnosed with renal cell carcinoma. The most common sites for metastasis are the lymph nodes, lung, bones, liver and brain. Metastatic RCC (mRCC) has a poor prognosis compared to other cancers. 5 year survival rate for for mRCC remains under 10% and 20-25% of patients remains unresponsive to conventional therapies. Aggressiveness of RCC and therapy resistance is associated with higher degree of oncogenic de-differentiation of tumor cells as evaluated by pathology grading and molecular features. Distinct population of cancer cells acquire features of adult stem cells with ability for self-renewal and tissue differentiation. These cells referred to as cancer stem cells (CSCs) sustain growth of the tumor, facilitates formation of metastases in distant organs. Within the tumor mass, CSCs reside in specific niches characterized by hypoxia and limited immune cells infiltration. CSCs develop broad array of

molecular means that confer them resistant to cancer therapies, including targeted therapies and immunotherapies.

MSc Imran Khan during his doctoral project undertook very ambitious task of identification of renal CSC followed by detailed functional and molecular characterisation. In the presented thesis, PhD candidate characterized to a detailed degree population of RCC cells characterized by the expression of CD105 surface marker (CD105+). The aim of the study was highly justified as CD105-positive cells have been previously shown to posses number characteristics of CSCs, including the ability to initiate tumor formation.

In the first series of experiments, MSc Imran Khan isolated the CD105+ cells from various primary and metastatic RCC cell lines. RT-qPCR revealed expression of stem cell-specific genes, including Oct-4 and Nanog. Both transcription factors are indispensable to sustain self-renewal of normal and malignant stem cells. Sorted CD105+ were shown to be positive for number markers that are shared with human mesenchymal cells, including: CD90, CD73, CD44, CD146, and alkaline phosphatase (activity). Series of functional experiments further revealed and confirmed stem cell-like phenotype of the CD105+ population, including ability to form three dimensional spheres. Finally, sorted CD105+ cells were using for transcriptome profiling using gene expression arrays. Bioinformatic analyses revealed deregulation of signalling pathways that are hallmarks of oncogenic de-differentiation. Results of these analyses evidently pointed towards abnormal activity of TGF-beta, Wnt/beta-catenine, as well as PI3K and Hippo signalling pathways. Importantly, phenotype of epithelial/mesenchymal transition, frequently associated with metastases formation was documented.

Obtained results were carefully discussed with the results presented in published literature. Data shown in MSc Khan thesis strongly support cancer stem cell phenotype of CD105+ cells that was previously reported by others. The gene profiling of CD105+ population followed by bioinformatic interpretation brings actual novelty and originality to the presented work. The author has performed full breadth of interpretation of the obtained molecular profiles. In the series of step-by-step analyses, one can learn the genes that are differentially expressed in the CD105+

cells. Next, using gene ontologies the author was able to group these genes in the meaningful biological mechanisms, including transcriptional networks and signalling pathways. Guided by the Ingenuity Pathway Analysis, the potential interactions between differentially expressed genes have been elegantly revealed.

In summary, I find the work presented by Imran Khan in his doctoral thesis not only interesting but importantly novel and original. The results of the experiments strongly support previous evidence that CD105+ cells are most likely the cancer stem cells in renal clear cell carcinoma. The detailed bioinformatic analyses of transcriptome profiling of these cells provides for the first time, insight into molecular wiring that drives oncogenic phenotype of renal CSCs. Presented work opens number of opportunities for further experimental work that would validate and extend described analyses. One could propose further functional validation of the stem cell-like phenotype of CD105+ cells using xenografts in animal models. Further development of patient-derived xenografts would be one of the possibilities. Understanding of the complexities of oncogenic transcriptional networks may ultimately pave the way for novel diagnostics and therapies for treatment of metastatic renal carcinomas.

The results presented in the Imran Khan's thesis were published last year in PLOS One, open access journal with broad international audience and recognizable impact. MSc Khan is the first author of this paper and I strongly believe also the major originator of this novel and interesting story. The minor drawback of the doctoral thesis was imperfect english writing, however this was effectively corrected in the published article.

I highly appreciate the presented work and kindly ask for the distinction.
Accordingly, I apply to the Scientific Board of the Military Institute of Medicine in Warsaw for admission of MSc Imran Khan to further stages of the doctoral dissertation.

Maciej Wiznerowicz

Dr hab. med. Maciej Wiznerowicz, prof. UMP