Título: UNDERSTANDING BLADDER CANCER THROUGH MOUSE MODELS AND EPIGENETIC TARGETS

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Resumen: Bladder cancer (BC) has been recognized as a clinically and molecularly heterogeneous disease with many genomic and epigenomic alterationsraising the question of which is the relevance of these genes in tumorigenesis. The development of several informative in vitro and in vivo models has been used to study bladder biology and have the potential to improve our understanding of BC progression, as well as its diagnosis and treatment. In this study, we have developed and characterized four genetically engineered mouse models of muscle invasive BC (MIBC) generated by the loss of two (Pten; Trp53) or four (Pten; Trp53; Rb1; Rbl1) tumor suppressor genes through the delivery of adenoviruses that express Cre recombinase in selected cell types (basal or lumimal cells of the urothelium) by the use of specific promoters. Molecular and transcriptomic analyses of tumors developed from these models revealed strong similarities to the human sarcomatoid subtype of BC, and some differences among the mouse models developed. Tumors originated in QKO mice were enriched in genes involved in the cell cycle and inflammation and showed more abundance in inflammatory infiltrates and necrosis, while those developed in DKO mice were mainly enriched in genes related with angiogenesis and epithelialmesenchymal transition and showed fewer immune infiltrates and necrosis, providing a useful framework for further studies. In addition, tumors originated from basal cells (K5 positive) had higher metastasis capacity than tumors originated in luminal cells (K20 positive) in both DKO and QKO models. We then extended the methods to develop transplantable murine MIBC cell cultures that allow serial transplantation in

immunocompetent syngeneic hosts. Subcutaneous syngeneic mouse models developed and characterized showed conservation of the parental primary tumor features, providing us with a useful tool for preclinical studies. With these tools, we have performed a proof-of-concept therapeutic approach analyzing the different response to CDK4/6 inhibition (Palbociclib) alone or in combination with an anti-PD-L1 (Avelumab) and related their response to different pathological conditions (cytokine expression, immune cell recruitment, etc.). We observed, in agreement with previous results of the group, that the CDK4/6 inhibitor is active in all the models, irrespective of their genetic characteristics (i.e., bearing or not functional Rb1 alleles), although the mechanisms of action appear to be different. We also observed cooperation between Palbociclib and Avelumab in tumor therapy with different immune effects depending on the model used.

Finally, to study the role of G9A or EZH2 genes in BC cells, knock-out cells were generated using two different human BC cell lines by CRISPR/Cas9 approaches. These engineered cells were used to express a wt or a mutant (catalytically inactive) form of the corresponding deleted gene. The modified cell lines (and the parental counterparts) were used for RNA-seq and ATAC-seq characterization. We determined that the G9a and EZH2 epigenetic modulators exert different roles in BC cell biology. G9a modulates the expression of immune-related genes, whereas EZH2 modulates the expression of proliferation/differentiation genes. In conclusion, the development of different mouse models, their use as preclinical tools for the evaluation of new therapeutic approaches, and the studies of epigenetic effectors in BC cell lines has allowed us to delineate relevant molecular pathways involved in the biology of this disease and novel approaches for its management.