Tumor Biology

HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY

Epithelial Transcription Factor FOXA1 Regulates Prostate Cancer Immune Response

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Background: While localized prostate cancer (PCa) can be mitigated by surgery and radiation, metastatic PCa remains a challenge to treat. Androgen deprivation therapies and androgen receptor (AR) pathway inhibitors are mainstay treatments for advanced PCa. Yet, resistance often develops leading to castration-resistant prostate cancer (CRPC). Forkhead Box A1 (FOXA1) is a pioneer transcription factor that plays pivotal roles in regulating AR activity and promoting epithelial differentiation. Studies have shown that FOXA1 is frequently downregulated in CRPC tumors. Congruently, FOXA1 loss is reported to induce aberrant AR signaling, epithelial-mesenchymal transition, and PCa de-differentiation. However, the role of FOXA1 in regulating PCa immune response, an area of much interest recently, has not been reported. CRPC has shown poor response to immune checkpoint inhibitors, due to its immunosuppressive nature. A better understanding of the tumor intrinsic mechanisms regulating PCa tumor immunity will inform the design of better targeted immunotherapeutic approaches. **Methods:** We performed RNA-seq, ChIP-seq, qPCR, western blot, and ELISA analyses to evaluate how FOXA1 regulates inflammatory response genes. We utilized an *in vitro* macrophage infiltration transwell assay, in which M2-like macrophages were added to the upper chamber and PCa cells were plated in the lower chamber, to examine how perturbations to PCa cells affect macrophage migration. Finally, we performed bioinformatic analyses of patient datasets to confirm the clinical relevance of FOXA1 repression of inflammatory genes in PCa. **Results:** Through integration of RNA-seq and ChIP-seq data, we uncovered a novel function of FOXA1 in suppressing inflammatory response pathways. In accordance, patient data analyses revealed that inflammatory response genes were upregulated in FOXA1-low PCa tumors. Mechanistically, we showed that FOXA1 proteins bound an intragenic enhancer of Hypoxia-inducible factor 1-alpha (HIF1A) gene to directly repress its expression, such that FOXA1 loss induced HIF1A upregulation. We further showed that Monocyte Chemoattractant Protein-1 (MCP-1/CCL2) became upregulated upon FOXA1 depletion in a HIF1A-dependent manner. This led to infiltration by immunosuppressive, tumor promoting M2-like macrophages. Inhibiting this HIF1A-CCL2 axis with a HIF1A inhibitor or CCL2 neutralizing antibody blocked macrophage infiltration. Future studies using immunocompetent mouse models are needed to confirm the effect of FOXA1 on macrophage infiltration *in vivo* and evaluate the preclinical potential of targeting the FOXA1-HIF1A-CCL2 axis in CRPC. **Conclusion:** This study proposes a novel role for FOXA1 loss in promoting macrophage infiltration via the HIF1A-CCL2 axis. Moreover, our findings suggest that targeting this axis may be a promising approach for the treatment of FOXA1-low CRPC tumors.

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Estradiol Augments the Production and Actions of Polymorphonuclear Cells to Promote Lymphangioleiomyomatosis (LAM) Progression

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Affecting almost exclusively women, lymphangioleiomyomatosis (LAM) is a rare lung disease characterized by slowly growing, estrogen-sensitive metastatic smooth muscle cell-like adenomas that result in cystic lung changes and loss of pulmonary function. LAM tumors are caused by mutations in either *TSC1* or *TSC2* genes that induces defective inhibition of the mTORC1 pathway, leading to increased mTORC1 activity and augmented cell proliferation. We have previously reported that estrogen ablation in our uterine-specific *Tsc2* knockout mouse, which grows tumors with characteristic LAM features and lung colonization potential, effects notable regression of tumors. Thus, estrogen is required for to maintain heightened mTORC1 activity and LAM-like tumor progression. Interestingly, the observed estrogen sensitivity *in vivo* is more markedly pronounced than that of our estrogen receptor-positive TSC2-null cells when stimulated with estradiol *in vitro*, suggesting that estradiol may act elsewhere—in mTORC1 independent manner—*in vivo* to promote LAM progression. Flow cytometry revealed large numbers of Ly-6C^{int} Ly-6G^{high} myeloid cells—polymorphonuclear cells or PMNs—in the blood and myometrial tumors of our uterine-specific *Tsc2*-null mice. Accordingly, we found that *Tsc2*-null tumors required PMNs for normal disease progression, as Gr-1 (Ly-6C/Ly-6G) depletion or inhibition of PMN recruitment reduced tumor growth. Therefore, we hypothesized that, in addition to direct effects of estrogen on tumor cells, estrogen might also stimulate tumor growth by promoting PMN production in the bone marrow and actions in the tumor microenvironment. Using bone marrow cultures, we found that estradiol is indeed a potent inducer of PMN production. This effect occurs equally in both male and female bone marrow. Employing both pharmacologic agents and bone marrow from ERα; knockout mice, we showed that ERα; is necessary for promoting a PMN fate for myeloid progenitors. Additionally, we have evidence implicating estrogen in the pro-tumorigenic function of PMNs co-cultured with TSC2-null cell lines. Overall, these data suggest that estradiol maybe facilitating cross-talk in LAM tumors, directly stimulating tumor cells while also promoting the production and actions of PMNs, which in turn promote tumor growth.

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HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY

Evaluation of a Nep-Score Threshold and the Derived Nep-D Score in Predicting Survival of Patients With Typical and Atypical Bronchial Carcinoids

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Background: Typical and atypical bronchial carcinoids (TBC and ABC) display a wide range of clinical presentations and may behave very differently. Survival prognostic markers are necessary to better define therapeutic strategies. AIM: verify that the NEP-Score, recently proposed as prognostic score, can be applied in a homogeneous TBC and ABC cohort and identify a derivate prognostic marker taking into account clinical and pathological characteristics at diagnosis. **Methods:** Age, site of primary tumor, primary tumor surgery, symptoms, Ki67, timing of metastases of 64 patients including TBC and ABC were evaluated to calculate the NEP-Score at the end of follow-up (NEP-T). We then assessed a derivative score considering the NEP-Score at diagnosis (NEP-D): this score does not consider the appearance of new metastases during follow-up. We then considered the patients that were alive or dead at the end of follow-up (EOF). A NEP-Score threshold to predict survival was investigated. **Results:** live patients at EOF displayed a mean NEP-T and mean NEP-D significantly lower as compared to those that were dead. A NEP-T threshold >138 significantly predicts survival. ABC relapsed more frequently as compared to TBC. Male gender as well as previous malignancy were negative prognostic factors for survival. **Conclusions:** We found that NEP-Score is applicable to a series of bronchial neuroendocrine neoplasms. In addition, we propose NEP-D as a simple, quick and cheap prognostic score that can help clinicians in decision making. Moreover, the use of a NEP-D threshold can predict NEN aggressiveness and may be used to define the best personalized therapeutic strategy. Furthermore we found additional prognostic factors that together with the NEP-Score could improve prognosis evaluation at diagnosis by using easily accessible information.

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HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY

Evaluation of the Octreotide Acetate Pen Injector in a Formative Human Factors Study

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Introduction: Subcutaneous injection of octreotide acetate is indicated to treat adults with acromegaly and diarrhea associated with carcinoid tumors or vasoactive intestinal peptide tumors. In this formative human factors study, we evaluated the readability and comprehension of the instructions for use (IFU) and ease of use of the octreotide pen injector. **Methods:** The study enrolled patients and healthcare practitioners who would be using the pen injector. The IFU contained a stepwise process with illustrations to detail injection administration and safe storage of the octreotide pen injector. Participants read the IFU and familiarized themselves with the device. Participants administered 2 unaided injections into skin-like pads. Injection success was defined as an attempt that delivered the correct dose into the pad. Each injection was evaluated by objective performance and subjective measures. Objective performance measures included assessment of steps necessary to deliver the correct medication dose and ensure user safety. Subjective measures included soliciting participant feedback on perceived success and difficulties administering a dose with the octreotide pen injector, as well as suggestions for improvements. Additional goals included evaluation of the IFU and octreotide pen injector usability aspects. **Results:** A total of 8 patients and 3 healthcare practitioners enrolled in the study. All (n = 11) participants successfully administered both injections, leading to an overall injection success rate of 100% across twenty-two injections. Subtask errors included participants priming the pen injector with the incorrect dose (n = 1) and not holding the injection button for 10 seconds after the injection (n = 2), but neither error resulted in dosing failure. Participant suggestions for improving the IFU included changes to the illustration of the plunger, reordering statements to clarify the priming process, and detailing how long to let the pen injector come to room temperature. **Conclusion:** Overall, participants felt the octreotide pen injector was easy to use and the instructions were clearly written and illustrated. Participant feedback and observations by moderators of the study led to recommendations for improvements to the clarity of the IFU.

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FOXO1 Mitigation of FOXL2C143W/SMAD3 Transcriptomic Landscape in a Model of Granulosa Cell Tumor.

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Background: Adult granulosa cell tumor (aGCT) is a rare type of stromal cell malignant cancer of the ovary. Postmenopausal genital bleeding is the main aGCT clinical sign which is attributed to estrogen excess driven by CYP19 upregulation. Typically, aGCTs that are diagnosed at an initial stage can be treated with surgery. However, recurrences are mostly fatal[1]. Current studies are focused on finding new molecular markers and targets that aim to treat the aGCTs recurrence. Between 95-97% of aGCTs harbor a somatic mutation in the FOXL2 gene, Cys134Trp (c.402C<G)[2]. A TGF-β pathway protein, SMAD3, was