has a 5-year survival rate of only 10% in 2020. Using human and mouse pancreatic cancer cells and RNA and protein expression analyses by RT-qPCR, ELISA, and western-blot, we identified that (i) GH upregulates specific ABC-transporter expressions in a drug-context specific manner, (ii) GH upregulates EMT transcription factors, (iii) GH activates specific oncogenic signaling pathways, and (iii) GH action increases cytochrome P450 members involved in hepatic drug metabolism. The GH antagonist, Pegvisomant, significantly inhibited these effects. Additionally, we confirmed the effects of these molecular changes by specific assays. For example, GH increases basement membrane invasion, viability of circulating tumor cells, and drug efflux; while inhibition of GHR by pegvisomant in pancreatic cancer cells reversed this aggressive tumor phenotype and sensitized the tumor cells to chemotherapy. Cell viability assays confirmed a decreased IC50 of gemcitabine, doxorubicin, and erlotinib in pancreatic cancer cells treated with pegvisomant and an increase in IC50 cells treated with GH. We further verified our results using in silico analyses of TCGA datasets for pancreatic cancer - which provided robust confirmation of our experimental findings. Presently we are validating our observation in nude mice with human pancreatic cancer cell xenografts. In conclusion, our *in vitro* results confirm that GHR antagonism can drastically sensitize human pancreatic cancer cells by blocking mechanisms of drug resistance, thus providing a valuable window for improved efficacy of available chemo- and targeted therapy.

Tumor Biology

HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY

Halting Retrograde Transport Excludes ErbB-2 From the Nucleus Abrogating Tumor Growth in Triple Negative Breast Cancer

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Triple negative breast cancer (TNBC) refers to a subtype of tumors with poor prognosis, devoid of hormone receptors and of membrane overexpression or gene amplification of ErbB-2. Due to its molecular heterogeneity, TNBC represents a major clinical challenge. In this regard, clinical biomarkers and targeted therapies remain elusive, and chemotherapy has been the standard of care for early and metastatic TNBC. ErbB-2, a member of the ErbB family of tyrosine kinase receptors, is a major player in the BC scenario. While it is a cell membrane-bound receptor, it migrates to the nucleus (NErbB-2) where it acts as a transcription factor or coactivator. We recently found that both the canonical (wild-type, WT) ErbB-2 and the alternative isoform c are located in the nucleus of TNBC, a scenario with an aggressive oncogenic potential. The route of intracellular transport from the plasma membrane to the trans Golgi network (TGN) and the endoplasmic reticulum (ER) is termed retrograde trafficking, and constitutes the pathway by which ErbB-2 migrates to the nucleus. The retrograde transport route is also hijacked by toxins and viruses to access the ER and exert their deleterious effects. Retro-2, a small molecule inhibitor, was shown to protect cells from toxin and virus effects by blocking their retrograde trafficking. Given the high levels of NErbB-2 in TNBC cells, we explored whether treatment with Retro-2 modulates localization of ErbB-2 and proliferation in TNBC.

We found that Retro-2 treatment decreased the levels of both WT ErbB-2 and isoform c in the nucleus of TNBC cells demonstrating that Retro-2 effects are not limited to a particular ErbB-2 isoform. Indeed, immunofluorescence assays revealed accumulation of ErbB-2 in the Golgi after Retro-2 treatment further preventing its sorting to the ER. We previously demonstrated that growth factors induce ErbB-2 migration into the nucleus in ErbB-2-positive BC cells. Consistently, we observed that Retro-2 prevents growth factor-induced NErbB-2 in ErbB-2-positive BC cells. Retro-2 treatment resulted in a dose-dependent decrease in cell proliferation in a panel of TNBC cells, whilst did not inhibit cell proliferation in the ErbB-2-negative MCF10A normal breast cell line. Moreover, disruption of retrograde transport by Retro-2 decreased the expression of cell cycle related NErbB-2 target genes (*i.e.* Erk5 and cyclin D1) therefore inducing cell cycle arrest at the G0/G1 phase. Most importantly, Retro-2 excluded ErbB-2 from the nucleus and abrogated tumor growth in preclinical models of TNBC.

Collectively, our findings reveal Retro-2, a non-toxic inhibitor of the retrograde transport route, as a candidate novel therapeutic agent for TNBC based on its ability to evict ErbB-2 from the nucleus and to abrogate TNBC growth.

Tumor Biology

HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY

Heterogeneous Nuclear Ribonucleoprotein K Is Involved in the Estrogen-Signaling Pathway

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Heterogeneous nuclear ribonucleoprotein K (hnRNPK) has been found in the nucleus, cytoplasm, and mitochondria. It is implicated in chromatin remodeling, transcription, splicing, and translation processes. Although hnRNPK has reportedly been associated with poor prognosis in colon cancer patients, it is beneficial in gastric cancer as it inhibits cancer cell proliferation. Expression of hnRNPK in ER (Estrogen receptor) -positive/PR (Progesterone receptor) -positive breast cancer was higher than other subtypes; however, the biological functions of hnRNPK in the ER-mediated signaling pathway have not been identified. In this study, we investigated the functions of hnRNPK in the estrogen-signaling pathway. We initially evaluated

hnRNPK expression upon treatment with estradiol (E2) and ICI 182,780 in ERα-positive breast cancer cell line MCF-7. This initial evaluation revealed that expression of hnRNPK was increased by E2 treatment but decreased by ICI 182,780 treatment. We further evaluated the effects of estrogen-signaling pathway in hnRNPK knockdown MCF-7 cells using siRNA, which revealed that hnRNPK knockdown decreased ERα expressions and ERα target gene TFF1 by E2 treatment. In addition, we examined the interaction between hnRNPK and ERα because hnRNPK has been reported to interact with several other proteins. These interactions were detected using immunoprecipitation and proximity ligation assay. We then immunolocalized hnRNPK in breast cancer and endometrial cancer. hnRNPK expression was significantly higher in ERα-positive cancer cells in both breast and endometrial cancers. In contrast, hnRNPK expression was significantly lower in Ki-67-positive breast cancer while being significantly higher in Ki-67-positive endometrial cancer. hnRNPK has been found to function differently, depending on the type of cancer (breast or endometrial) that it is expressed in. However, further studies are required to clarify the clinical significance of hnRNPK in breast and endometrial cancer patients.

Tumor Biology

HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY

High Expression of Nucleobindin-2 Is Associated With Poor Prognosis in Gastric Cancer

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Nucleobindin-2 (NUCB2) is a 396-amino acid protein, cleaved into the N-terminal nesfatin-$1_{1\text{-}82}$, nesfatin-$2_{85\text{-}163}$ and the C-terminal nesfatin-$3_{166\text{-}396}$. NUCB2 contains a signal peptide, a leucine zipper structure, two Ca^{2+} binding EF-hand domains, and has a wide variety of basic cellular functions. NUCB2 is also a precursor protein of nesfatin-1, which was originally identified in hypothalamic nuclei, and which is a regulatory factor involved in the central control of food intake and energy balance. There are several reports indicating that NUCB2 is also expressed in various human peripheral tissues. Moreover, recent studies have reported that high levels of NUCB2 mRNA and protein are a potent prognostic factor for prostate cancer, endometrial carcinoma, and breast cancer. NUCB2 was also identified as a potential tumor antigen eliciting autoantibody responses in 5.4% of gastric cancer patients but not in the healthy individuals. However, theclinicopathological significance of NUCB2 expression in gastric cancer has still not been elucidated. Therefore, we examined NUCB2 expression in a large number of gastric cancer patients, using immunohistochemistry, to explore its clinicopathological significance. To explore this, we aimed to investigate the NUCB2 expression in gastric cancer tissues and adjacent non-tumor tissues and its potential relevance to clinicopathological factors and prognosis using immunohistochemistry analysis. In our study, NUCB2 level in gastric cancer tissues was higher than in non-tumor tissues. A high expression of NUCB2 is significantly associated with tumor depth, lymph node metastasis, lymphatic invasion, venous invasion and clinical stage. Furthermore, the expression level of NUCB2 protein was independent predictor of progression-free survival. In summary, NUCB2 might play a crucial role in gastric cancer development and could serve as an independent predictor of prognosis of gastric cancer patients.

Tumor Biology

HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY

Hormonal Regulation of Semaphorin 7a Promotes Therapeutic Resistance in Breast Cancer

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Background: The majority of all breast cancers (BC) are estrogen receptor positive (ER+). While ER-targeting endocrine therapies have improved patient survival, many of these tumors develop drug resistance and recur within 20 years. Therefore, novel targets are needed to predict for recurrence and to treat recurrent ER+BC. Previous reports describe a tumor-promotional role for Semaphorin 7A (SEMA7A) in ER- disease; yet, the role of SEMA7A in ER+ disease is poorly characterized. **Hypothesis:** SEMA7A promotes cell survival and drug resistance in ER+ BC. **Methods:** We overexpressed SEMA7A in ER+ BC cells, then used the ER-targeting agents tamoxifen and fulvestrant to test how SEMA7A-expressing cells respond to endocrine therapy. In vitro, we used proliferation and cell survival assays. In vivo, we implanted ER+ BC cells, then treated the animals with fulvestrant to measure how SEMA7A affects tumor growth and metastasis. We also utilized drug resistant cells, which have high endogenous SEMA7A levels, to measure markers of stemness and multi-drug resistance via flow cytometry. **Results:** We first found that SEMA7A expression correlates with decreased relapse free survival in patients with ER+BC who received endocrine therapy (Kmplotter; p=0.042). We also observe that SEMA7A is hormonally regulated in ER+BC, but its expression does not uniformly decrease with endocrine therapy agents. Instead, long term estrogen deprivation and ER-targeting drug treatments increase SEMA7A expression, likely through the action of other hormone receptors such as the androgen receptor, which also increases with long term estrogen deprivation. Further, in ER+ cell lines, overexpression of SEMA7A promotes in vitro growth in the face of estrogen-deprivation, tamoxifen, or fulvestrant treatments. In vivo, SEMA7A promotes fulvestrant resistance in the primary tumor and induces lung metastases. Finally, we report that pro-survival signaling is a therapeutic vulnerability of ER+SEMA7A+ tumors. **Conclusion:** These studies describe that SEMA7A promotes drug resistance in ER+ BC. We propose that targeting pro-survival signaling may prove