Western Blot analysis. 16 tumours were obtained during neurosurgery, 8 metastatic and 8 recurrent tumours of the same patient, and immediately frozen using liquid nitrogen. The proteins were extracted using RIPA lysis buffer. Western Blot was performed and detection followed via peroxidase linked secondary antibodies. RESULTS: MTH1 expression was shown to be up-regulated in brain metastases (1,442+/-0,6374) versus normal brain tissue (0,4133+/-0,277). However, in the recurrent tumour of the brain metastases, MTH1 was not expressed in a significantly higher amount compared the controll tissue and less than in the brain metastases (0,6941+/-0,4146). CONCLUSION: The high expression of MTH1 in cerebral metastases is not uncommon for many cancers and thus presents a therapeutic target for MTH1-inhibitors, provided these are able to cross the blood brain barrier. Comparison to the primary melanoma tumour would be useful in showing significant differences of the metastases. Lower levels of MTH1 in recurrent brain metastases may suggest dedifferentiation from the original metastases. It may present a target for specific treatment of brain metastases of melanoma.

BSCI-14. TGLI1 IS AN ACTIONABLE THERAPEUTIC TARGET IN BREAST CANCER BRAIN METASTASES

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Breast cancer is the second leading cause of brain metastases in women; patients with breast cancer brain metastasis (BCBM) survive a median of 14.1 months following diagnosis. Cancer stem cells are thought to be one of the driving forces behind distant metastasis, treatment resistance, and late-stage recurrence. Despite advances made in understanding breast cancer stem cells (BCSC), it remains challenging to effectively target BCSC underscoring the need to identify and inhibit novel mediators of BCSC for treating BCBM patients. The hedgehog-smoothened pathway is an important mediator of breast cancer stem cells (BCSC); however, FDA-approved therapies targeting smoothened have demonstrated limited clinical efficacy in breast cancer. Truncated glioma-associated oncogene homolog 1 (tGLI1) was discovered in our laboratory as an alternative GLI1 splice variant that functions as a tumor-specific gain-of-function transcription factor and terminal effector of the hedgehog pathway. Our laboratory recently reported that tGLI1 promotes preferential metastasis to the brain in breast cancer by activating BCSC and astrocytes in the tumor microenvironment (Oncogene 39:64–78, 2020). tGLI1 knockdown abrogated BCBM, providing the rationale to therapeutically target tGLI1. This study aimed to determine if tGLI1 can be therapeutically targeted. Cell-based chemical screens followed by validations demonstrated that ketoconazole, an FDA-approved azole antifungal, and novel derivatives specifically inhibit tGLI1 leading to suppression of BCSC *in vitro* and BCBM *in vivo*. Mechanistic studies suggest that KCZ-dependent cell kill is, in part, mediated through downregulation of tGLI1 target genes *OCT4*, *Nanog*, and *VEGFA*. Based on these data, we opened a window-of-opportunity study in patients with BCBM to determine if ketoconazole penetrates the blood-brain barrier (BBB) and alters tGLI1 signaling in humans (NCT03796273). Preliminary sample analysis demonstrates ketoconazole crosses the blood-tumor barrier and that tGLI1 expression correlates with tGLI1 signaling in resected samples. Collectively, these data establish tGLI1 as an actionable target for BCBM.

BSCI-15. OSTEOPONTIN PLAYS A CRUCIAL ROLE IN INVASIVENESS OF TRIPLE NEGATIVE BREAST CANCER CELLS IN THE CONTEXT OF HUMAN MICROGLIA

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The triple-negative breast cancer (TNBC) is the most malignant among breast cancers and has the high risk of developing metastasis into the brain. Metastases of breast cancers are increasing and pose a clinical challenge as the current treatments are not effective due to the unique brain microenvironment for metastatic breast cancer cells. While the contribution of brain macrophages to the formation of the metastatic niche is established, factors responsible for the crosstalk between cells remain elusive. *SPP1* encoding a secreted phosphoprotein 1 (ostepontin) is highly overexpressed in malignant breast cancers. We evaluated the role of SPP1 in invasion and metastasis of human breast cancer cells. We found the increased invasion of triple-negative MDA-MB-231 (MDA-231) cells in the presence of human microglial HMSV40 cells. Using Western blot analysis demonstrated the elevated levels of focal adhesion kinase (FAK) and signal transducer and activator of transcription 3 (STAT3) in MDA-231 cells in co-cultures. Moreover, blocking SPP1 and integrin interactions with the synthetic RGD peptide, efficiently diminished both basic and microglia-induced invasion of MDA-231. To assess the role of SPP1 in cell invasion, we established the MDA-231 cells with knocked-down SPP1 expression using shRNA (shSPP1). Interestingly, the shSPP1 cells were unresponsive towards HMSV40 microglia. We have previously found that an antibiotic minocycline reduces *SPP1* expression in glioma cells. We performed cell toxicity studies on 4 breast cancer cell lines and various non-malignant cells. All tested malignant cancer cells were more sensitize to minocycline than non-cancerous cells and breast cancer cells derived from TNBC were the most susceptible. Altogether, we demonstrate that microglia support invasion of breast cancer cells via SPP1/osteopontin triggering the integrin signalling, and minocycline by downregulating *SPP1* expression may reduce both basic and microglia-induced cancer invasion. Therefore, we purpose that minocycline could be a new therapeutics targeting metastatic brain cancers.

BSCI-16. OLFACTORY RECEPTOR 5B21 DRIVES BREAST CANCER METASTASIS

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Olfactory receptors (ORs), responsible for the sense of smell, play an essential role in physiological processes (even outside the nasal epithelium) and cancer. In breast cancer, however, the expression and role of ORs remain understudied. We examined the significance of ORs transcript abundance in breast cancer metastasis to different tissues including the brain, bone, and lung. While we found 20 OR genes to be differentially expressed in different metastasis versus primary tumor, OR5B21 displayed high relation with all metastases. Knockdown of OR5B21 significantly decreased the invasion and migration of breast cancer cells in culture as well as metastasis to different organs including the brain, *in vivo*. On the other hand, overexpression of OR5B21 in the primary cells had the opposite effect. Mechanistically, OR5B21 was associated with epithelial to mesenchymal transition through STAT3/NFkB/CEBPβ signaling pathway. We propose OR5B21 (and potentially other ORs) as a novel oncogene contributing to breast cancer metastasis, and as a potential target for therapy.

BSCI-17. TARGETING SIRPA AS A THERAPEUTIC STRATEGY FOR THE TREATMENT OF BREAST CANCER BRAIN METASTASIS

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Triple-negative breast cancer (TNBC) is a highly aggressive subtype of breast cancer characterized by the lack of druggable targets and an incidence of brain metastasis from the primary site of approximately 35%. There is no standard treatment for managing brain metastasis associated with TNBC; therefore, new strategies are urgently needed to overcome disease mortality. The CD47/SIRPα signaling pathway is implicated in tumor progression due to bypassing innate and adaptive immune surveillance. Most strategies targeting this pathway focus on targeting the receptor CD47; however, targeting SIRPα as a potential strategy to mitigate tumor burden remains understudied. Analysis of gene expression database shows that SIRPα expression is significantly elevated in invasive breast cancer when compared to primary. Furthermore, single-cell data indicates that SIRPα is expressed in basal epithelial cells in TNBC tumors aside from the myeloid compartment. Our immune staining against SIRPα in patient biopsies shows a five-fold increase in SIRPα expression in metastatic brain tumors compared to the primary lesions. Therefore, targeting SIRPα may be a new immunotherapeutic strategy to treat breast cancer brain metastases. Anti-SIRPα treatment of mice bearing brain metastatic 4T1br3 orthotopic tumors showed reduced tumor volume and tumor weight by over 50% compared to isotype control-treated mice. Furthermore, in a model of intracardial brain metastasis, treatment with SIRPα antibody was associated with a 60% increase in survival compared to isotype control-treated mice. RNA sequencing of tumors indicated that SIRPα blockade is associated with a reduction in genes linked to mitochondrial respiratory chain and increases in negative regulation of the cell cycle. Furthermore, in vitro SIRPα targeting enhanced the cell-mediated cytotoxicity of microglia against 4T1Br3 breast cancer cells. This suggests that SIRPα blockade may influence both tumor and innate immune cells to limit brain metastatic breast cancer growth and enhance survival.