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heat shock response (HSR) by mEHT, which can result in thermotolerance. We hypothesized that downregulation of HSF1 gene sensitizes the transfected tumor cells to mEHT and reduce tumor growth.

Material and Methods

A Balb/C isogenic murine TNBC cell line (4T1) was used. HSF1 CRISPR/Cas9 lentiviral knockdown or wild type 4T1 cells were inoculated into mammary gland's fat pad. Further, wild type tumors were treated with the HSF-1 inhibitor KRIBB11, for 8 days. Four mEHT treatments were performed every two days and the tumor growth was followed by ultrasound and caliper. Tumor destruction histology and molecular expression changes were assessed.

Results and Discussions

Reduction of tumor size and weight, and enlargement of tumor destruction area were observed in HSF1-KO mEHT-treated mice vs HSF1-KO Sham (HSF1-KO: 43.66 mg ± 20.09 mg; 84.45% ± 15.66% vs Sham: 110.0 mg ± 44.54 mg; 32.1% ± 14.52%, respectively) and Empty Vector mEHT-treated (89.49 mg ± 24.8 mg; 77.24% ± 7.34%, respectively). HSF1 mRNA level was significantly reduced in the KO group (Sham: 0.006180 ± 0.0006644; mEHT-treated: 0.005832 ± 0.001073) when compared to Empty Vector group (Sham: 0.01330 ± 0.002487; mEHT-treated: 0.01732 ± 0.004167). CRISPR/Cas9 lentiviral construct was able to diminish the induction of HSP70 mRNA expression (mEHT-treated: 0.01046 ± 0.005662) when compared to Empty Vector mEHT-treated group (0.02273 ± 0.01385). Immunohistochemistry confirmed the molecular data. Combined therapy of mEHT and KRIBB11 significantly reduced tumor weight (160.3 mg ± 33.26 mg) further compared to monotherapy (mEHT: 236.8 mg ± 46.42 mg; KRIBB11: 312.3 mg ± 41.45 mg).

Conclusion

Combined mEHT-therapy with HSF1 inhibition can be a possible new strategy of treating TNBC with a great translational potential.

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EACR23-0430

Non-steroid anti-inflammatory treatment enhances the efficacy of modulated electro hyperthermia on triple negative breast cancer and melanoma cancer models in vivo

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Introduction

Modulated electro-hyperthermia (mEHT) is an advanced option in the hyperthermia field, applying a 13.56 MHz radiofrequency electromagnetic current to induce tumor-specific damage. This study investigates the mEHT-induced molecular effect and the potential of combination non-steroid anti-inflammatory drugs (NSAIDs) to enhance its anti-tumor effects in 4T1 triple-negative breast cancer (TNBC) and B16F10 melanoma mouse models.

Material and Methods

4T1 TNBC and B16F10 melanoma cell lines were injected into Balb/C and C57BL/6 mice, respectively. They have been treated according to the protocol with only mEHT or mEHT combined with non-selective COX-inhibitors (Aspirin) or selective COX2 inhibitors (SC236). Tumor volume was monitored by ultrasound and a digital caliper. At the end of the experiments, mice were euthanized and tumors excised for molecular studies.

Results and Discussions

Our previous multiplex studies, demonstrated that mEHT induced a local acute phase response (APR) in TNBC. Here we report that mEHT monotherapy stimulates local IL1-beta and IL6, and consequently cyclooxygenase 2 (COX 2) production. These effects could be considered as part of a self-defensive, wound-healing reaction of the tumor to protect itself from the mEHT-induced stress. In the present study, we combined mEHT with non-steroid anti-inflammatory drugs (NSAIDs), the non-selective (Aspirin), or the selective COX2 inhibitor (SC236) in vivo. All of these therapies have already demonstrated antitumor effects in various cancer models as monotherapies. Here we demonstrate that NSAID treatment synergistically increased the effect of mEHT in 4T1 TNBC. Tumor weight and tumor volume (measured by ultrasound and a digital caliper) were lowest, and the tumor destruction ratio (TDR) was the highest in the combination treated (NSAID + mEHT) groups. Tumor damage was accompanied by a significant increase in cleaved caspase-3 (cC3), suggesting an important role for apoptosis. Similarly, in the B16F10 melanoma model, lungs nodules were significantly less in mice treated with mEHT + Aspirin.

Conclusion

NSAIDs effectively enhance the mEHT anti-tumor effect in TNBC and melanoma cancer models; they increase tumor destruction, where apoptosis may play a role. Disecting the exact molecular mechanisms further is under our current investigation.

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EACR23-0484

Novel functional immunoassay for identification of multidrug resistance markers in non-small cell lung carcinoma patient-derived cells

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Introduction

Multidrug resistance (MDR) significantly hampers non-small cell lung carcinoma (NSCLC) drugs' efficacy. To evaluate the contribution of MDR markers to anticancer drugs' sensitivity, we performed pharmacological screening on patient-derived NSCLC cells *ex vivo* and assessed the expression of MDR markers in cancer and stromal (non-cancer) cells.

Material and Methods

Primary patient-derived cultures were established from the NSCLC resections. After short-term culturing (2-3 weeks), a mixed population of cancer and non-cancer cells were treated with 8 chemotherapeutics (cisplatin, carboplatin, paclitaxel, docetaxel, etoposide, vinorelbine, gemcitabine, and pemetrexed). The maximum concentration reached in human plasma to which the patient is exposed during therapy (Cmax) was set as an upper limit and four lower concentrations were also applied during the study. Immunofluorescence assay enabling discrimination of epithelial cancer cells positive to a cocktail of antibodies against cytokeratin 8/18 vs. negative mesenchymal non-cancer cells was conducted using high-content imager ImageXpress Pico (Molecular Devices) with CellReporterXpress 2.9 software. Within the same immunoassay, MDR markers (ABCB1, ABCC1, and ABCG2) were analyzed by corresponding antibodies.

Results and Discussions

Among all tested compounds, only gemcitabine increased the number of positive cancer cells to all MDR markers in all investigated primary cell cultures. Pemetrexed did not change the number of MDR-positive cancer cells. In a patient sample IIIA stage bearing EGFR mutation (L858R), the number of positive cancer cells to all MDR markers increased upon treatment with cisplatin, carboplatin, paclitaxel, docetaxel, etoposide, vinorelbine, and gemcitabine. Stromal (non-cancer) cells mainly followed the pattern of MDR observed in cancer cells.

Conclusion

Novel functional immunoassay can provide valuable information about the sensitivity of NSCLC to different drugs and possible treatment outcomes based on the expression of MDR markers.

EACR23-0531

Long-term gonadal effects of in-utero chemotherapy exposure

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Introduction

Pregnancy-associated cancer represents a unique clinical scenario, which requires a delicate balance between risks and benefits for the integrity of both mother and fetus. In case of cancer diagnosis during in the 1'st trimester, termination of the pregnancy is advised. When cancer is diagnosed during 2'nd and 3'd trimester, several classes of chemotherapy are allowed while doxorubicin (DXR) is the main chemotherapy used during pregnancy. Current clinical evidence regarding safety of chemotherapy administration during pregnancy refer mainly to cardiac and neurocognitive effects, there is lack of data regarding the impact on gonadal reserve. Moreover, since gonadogenesis occurs during pregnancy the impact of chemotherapy may be critical. Here we aimed to further evaluate the long-term male reproductive effects of in utero exposure to chemotherapy in a mouse model.

Material and Methods

Pregnant ICR mice were treated once with DXR (10mg/kg) or saline on day E12.5, litter was followed longitudinally for several systematic outcomes. Gonadal outcomes were evaluated in male offspring at three time-points: Day0 (birth), 10weeks (pubertal) and 6 months (full puberty). Mice were sacrificed and testes were processes, sperm extracted and blood drawn for Anti-Mullerian Hormone (AMH). Immunohistochemistry was employed to evaluate morphological changes, gonadal markers and apoptosis. Sperm was processed for RNASeq.

Results and Discussions

At birth in–utero DXR-exposed mice resembled the control mice in terms of histological appearance (condensed, organized spermatogonial filled seminiferous tubules). Nevertheless, at 10 weeks seminiferous tubules architecture appeared mildly disrupted in the exposed mice that dramatically worsened at 6-month age. Moreover, sperm count was reduced in the exposed mice compared to controls. DXR-exposed mice displayed an increased AMH levels, indicating an abnormal AMH regulation that may be derived from a toxic effect on supporting Sertoli cells. Sperm RNAseq of full pubertal exposed mice revealed significant profile of genes associated with spermatogenesis, sperms mobility, maturation and differentiation of spermatozoa compared with control mice.

Conclusion

Our study is the first to evaluate male reproductive outcomes of in-utero exposure to chemotherapy. Our results indicate that following exposure to chemotherapy in 2'nd trimester there is a latent effect manifested by a significant disruption of seminiferous tubules architecture, inferior sperm counts and differential gene expression pattern.

EACR23-0575

Patient-derived gastric tumour organoids recapitulate pivotal tissue molecular features making them useful for functional precision medicine.

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Introduction