INHIBITION OF LUNG CARCINOMA GROWTH AND PULMONARY METASTASIS WITH DIMATE AS SINGLE AGENT AND IN COMBINATION WITH CISPLATIN

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Introduction Aberrant cell proliferation in NSCLC causes an aberrant redox state that leads to the production of toxic reactive species and aldehydes. To keep oxidative stress until a threshold, above which oxidative damage can be detrimental to cell viability, cancer cells must actively upregulate multiple anti-oxidant systems. The aldehyde dehydrogenase (ALDH) gene superfamily encodes enzymes that are critical for certain life processes and detoxification of numerous endogenous and exogenous aldehyde substrates, including pharmaceuticals and environmental pollutants.

Material and methods In this study, a meta-analysis was conducted based on multiple microarray data from The Cancer Genome Atlas (TCGA) and gene expression omnibus (GEO) repositories, spanning lung adenocarcinoma and lung squamous cell carcinoma datasets. Twenty-six NSCLC cell lines with different oncogenic driver alterations were used to analyse the molecular and cellular consequences of ALDHs inhibition. Preclinical studies with orthotopic xenografts of NSCLC and lung metastatic breast cancer were performed to evaluate the effect of the inhibition of ALDH class 1 and class3 activity on tumour growth.

Results and discussions Here, we found that increased expression of aldehyde dehydrogenase isoenzymes ALDH1A1, ALDH1A3 and ALDH3A1 in human NSCLC tumours has a strong impact on chemotherapy resistance and patient overall survival, correlating with poor prognosis. We showed that inhibition of class 1 and class 3 ALDH activity with the novel irreversible ALDH1/3 inhibitor DIMATE suppresses tumour growth in orthotopic human lung cancer xenograft model and inhibits lung metastasis of human lung cancer in athymic nude mice. Accumulation of HNE-protein adducts and depletion of intracellular GSH are main responsible of DIMATE-inhibition dependent manner.

Conclusion Targeting the detoxification machinery of ALDHs constitutes a novel therapeutic avenue for NSCLC. Patients with increased expression of ALDH1 or ALDH3 might greatly benefit from a combination therapy that include drugs interfering with the activity of these enzymes to overcome patient-specific drug resistance.

TARGETING MUTANT P53 WITH COTI-2: A NEW APPROACH FOR THE TREATMENT OF PATIENTS WITH TRIPLE-NEGATIVE BREAST CANCER?

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Introduction The identification of a targeted therapy for triple-negative breast cancer (TNBC) is one of the most urgent needs in breast cancer therapeutics. Since the p53 gene is mutated in approx. 80% of TNBC tumours, it is an attractive target. COTI-2 is a clinical stage, small molecule which claims to target p53. The aim of this study was to investigate COTI-2 as a new treatment for TNBC.

Material and methods Cell viability was determined by MTT assay. p53 protein levels were quantified by ELISA and immunofluorescent staining. P53 binding kinetics were measured by Surface Plasmon Resonance. Apoptosis was measured using Annexin V-FITC Apoptosis Detection Kit. Caspase 3/7 was measured by CellEventCaspase-3/7 Green Flow Cytometry Assay Kit. CI values were calculated using CalcuSyn software.

Results and discussions Using a panel of 18 breast cell lines, TNBC cell lines were more responsive to COTI-2 than non-TNBC cells (p=0.04). Lower IC50 values for COTI-2 were found in p53 mutant vs p53 WT cells (p=0.001). Additionally, the higher the endogenous p53 protein, the more sensitive the cell line was to COTI-2 (p=0.035, r=-0.51, n=18). By staining with antibodies specific for folded WT p53 (PAb1620) or unfolded mutant p53 (PAb240), we showed that COTI-2 can induce refolding of mutant p53. Moreover, by SPR, we showed that COTI-2 binds to fl-mut-p53 protein, in a concentration dependent manner.

In addition to inhibiting proliferation, COTI-2 induced apoptosis in a concentration dependent manner. Furthermore, inhibition of caspase activity with Z-VAD-FMK reduced apoptosis, suggesting that COTI-2 induces caspase-dependent apoptosis. Accordingly, COTI-2 induced a significant increase in caspase 3/7.

In an effort to enhance response, COTI-2 was combined with a number of cytotoxic agents. Highly synergistic growth inhibition, i.e. CI <1, was found when COTI-2 was combined with doxorubicin in 6 different cell lines. In addition, COTI-2 plus docetaxel or eribulin was synergistic in 4/6 cell lines, plus carboplatin was synergistic in 3/6, while plus cisplatin was synergistic in 2/6. Finally, we compared response to COTI-2 with that of APR-246, the best studied p53 reactivating compound. Overall, the mean COTI-2 IC50 value was 64 fold lower than that for APR-246 (p=0.0006). Furthermore, no
correlation was seen between response to COTI-2 or APR-246, suggesting that the compounds act differently in inhibiting cell growth.

Conclusion We conclude that targeting mutant p53 with COTI-2 is a potential new approach for treating p53-mutated TNBC.

Introduction Triple-negative breast cancers (TNBCs) are a heterogeneous group of aggressive tumours lacking oestrogen and progesterone receptors and HER2 receptor, thus excluding the possibility of using targeted therapy against these proteins. Mesenchymal-like (ML) subtype, characterised by a stem-like, undifferentiated phenotype, is more invasive and metastatic than other TNBC subtypes and has a strong tendency to form vascularogenic mimicry (VM). Recently, platelet derived growth factor receptor β (PDGFRβ) has been shown to play a role in VM of TNBC. Regrettably, therapies targeting PDGFRβ with tyrosine kinase inhibitors are not effective in treating TNBCs, thus developing new strategies to target PDGFRβ in TNBC patients is crucial to improve their chances of survival. Here, we describe the characterisation of the Gint4.T anti-PDGFRβ nuclease-resistant RNA aptamer as a highly efficacious theranostic tool for imaging and suppression of ML TNBC metastases.

Material and methods Immunohistochemical analyses on a human TNBC tissue microarray was performed to correlate PDGFRβ expression with clinical and molecular features of different subtypes. Functional assays were conducted on PDGFRβ-positive ML BT-549 and MDA-MB-231 cells to investigate the effect of Gint4.T in interfering with cell growth in 3D conditions, migration, invasion and VM formation. Gint4.T was conjugated with near-infrared (NIR) fluorescent VivoTag-S680 and its binding specificity to receptor was confirmed both in vitro (confocal microscopy and flow cytometry analyses of TNBC cells) and in vivo (fluorescence molecular tomography in mice bearing TNBC xenografts). MDA-MB-231 cells were i.v. injected in nude mice and Gint4.T-NIR was used to detect lung metastases in mice untreated or i.v. injected with Gint4.T or a scrambled aptamer.

Results and discussions The expression of PDGFRβ was observed in human TNBC samples characterised by higher metastatic behaviour. Treatment of TNBC cell lines with Gint4.T aptamer blocked their invasive growth and vascularogenic properties in 3D culture conditions, and strongly reduced cell migration/invasion in vitro and metastases formation in vivo. The Gint4.T-NIR was able to specifically bind to TNBC xenografts and detect lung metastases in vivo. Therefore, the aptamer revealed a high efficacious theranostic tool for imaging and suppression of TNBC metastases.

Conclusion These studies indicate PDGFRβ as a new biomarker for ML and metastatic TNBC subtype and propose a novel targeting agent for the diagnosis and treatment of metastatic TNBCs.