oxidative stress as a common denominator. Such mechanistic understanding could further help to design pre-clinical and clinical studies.

235 Schisandrin B prevents doxorubicin-induced chronic cardiotoxicity and enhances its anticancer activity in vivo

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Background: To mitigate the cardiotoxicity of anthracycline antibiotics without compromising their anticancer activities is still an issue to be solved. We previously demonstrated that schisandrin B (Sch B) could protect against doxorubicin (Dox)-induced acute cardiotoxicity via enhancing cardiomyocytic glutathione redox cycling that could attenuate oxidative stress generated from Dox. In this study, we attempted to prove if Sch B could also protect against Dox-induced chronic cardiotoxicity, a more clinically relevant issue, without compromising its anticancer activity.
Materials and Methods: Rat was given intragastrically either vehicle or Sch B (50 mg/kg) two hours prior to i.p. Dox (2.5 mg/kg) weekly over a 5-week period with a cumulative dose of Dox 12.5 mg/kg. At the 6th and 12th week after last dosing, rats were subjected to cardiac function measurement, and left ventricles were processed for histological and ultrastructural examination. Dox anticancer activity enhanced by Sch B was evaluated by growth inhibition of 4T1, a breast cancer cell line, and S180, a sarcoma cell line, in vitro and in vivo.
Results: Pretreatment with Sch B significantly attenuated Dox-induced loss of cardiac function and damage of cardiomyocytic structure. Sch B substantially enhanced Dox cytotoxicities toward S180 in vitro and in vivo in mice, and increased Dox cytotoxcity against 4T1 in vitro. Although we did not observe this enhancement against the implanted 4T1 primary tumour, the spontaneous metastasis to lung was significantly reduced in combined treatment group than Dox alone group.
Conclusion: Sch B is capable of protecting Dox-induced acute and chronic cardiotoxicity and enhancing its anticancer activity. To the best of our knowledge, Sch B is the only molecule ever proved to function as a cardioprotective agent as well as a chemotherapeutic sensitizer, which is potentially applicable for cancer treatment.

236 Blockade of fatty acid synthase affects phosphatidylinositol-3 kinase signaling in ovarian cancer by ubiquitin-mediated degradation of downstream effector kinases

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Ovarian carcinoma is fourth leading cause of cancer death in women and accounts for highest mortality of all gynecological malignancies. The phosphatidylinositol-3 kinase (PI3K) cascade controls proliferation, differentiation, tumourigenesis, angiogenesis and apoptosis. Many ovarian carcinomas harbor aberrations within the PI3K pathway. Amplification of PI3K is observed in ~40% of ovarian carcinomas and cell lines. The PI3K downstream target AKT phosphorylates mTOR, which is hyperactivated in many cancers. mTOR activates S6 via p70S6K, which is frequently activated in ovarian cancer. S6 protein being a component of the 40S ribosomal subunit is involved in translation control. mTOR also phosphorylates eukaryotic translation initiation factor 4E (eIF4E) inhibitor binding protein 1 (4EBP1). Phosphorylated 4EBP1 dissociates from eIF4E and activates cap-dependent mRNA translation. In addition, many ovarian carcinomas harbor aberrations of the ErbB receptors ErbB1 (EGFR; 55%) or ErbB2 (HER2/neu; 35%), respectively. Importantly, PI3K signaling plays crucial roles in transmitting ErbB-derived signals and stimulating cancer growth. Irrespectively, clinical studies yet reveal that monotherapies with ErbB1 or ErbB2 inhibitors or antibodies are largely inefficient in ovarian carcinomas. Therefore, additional molecular targeting strategies are urgently needed. Fatty acid synthase (FASN) being overexpressed in ~80% of ovarian carcinomas is a marker for poor prognosis. It supports formation of lipid rafts in the plasma membranes, which accommodate transmembrane growth factor receptors incl. ErbB proteins. Thereby, FASN facilitates signal generation at the cell membranes. Most importantly, inhibition of FASN delays disease progression of ovarian carcinoma xenografts. Recently, we reported that the FASN inhibitor C75 downregulates ErbB1 and ErbB2 in ovarian cancer and sensitizes the cells against ErbB targeting drugs (Grunt et al., BBRC, 385, 454). We now demonstrate that C75 abrogates A2780 ovarian cancer cell growth. This correlates with silencing of PI3K downstream signaling as evidenced by reduced phosphorylation of AKT, mTOR, p70S6K and 4EBP1 in Western blot analyses, which is caused by both specific protein dephosphorylation/deactivation and by ubiquitin-mediated proteasomal degradation of these PI3K effector proteins. In contrast, specific phosphorylation/activation of the mitogen-activated protein kinase ERK1/2 is increased, although ERK1/2 steady-state levels are concurrently decreased by C75. In comparison, the PI3K inhibitor LY294002 blocks phosphorylation while concurrently upregulating steady-state levels of AKT, mTOR, p70S6K, and 4EBP1, and it activates ERK1/2. This suggests (i) that PI3K/AKT normally cross-inhibits ERK1/2, which can be abrogated by silencing of PI3K/AKT, and (ii) that PI3K, but not ERK1/2, signaling is crucial for growth arrest of ovarian cancer cells. Notably, our data demonstrate for the first time that C75-mediated silencing of PI3K signaling is caused by reduced phosphorylation and diminished protein stability due to increased ubiquitination and proteasomal degradation. Thus, C75 provides additive anticancer action, when compared to the PI3K inhibitor LY294002, which directly targets PI3K and downstream signaling, but does not stimulate effector protein degradation. In summary, FASN represents a promising anticancer drug target, which should be further developed for clinical use in ovarian carcinoma. Supp. 'Med.-Wiss. Fonds Bürgerm. Wien'.

237 Splice variant profiling in relation to tamoxifen resistance in breast cancer

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Tamoxifen, a selective estrogen receptor modulator (SERM), is commonly applied to treat estrogen receptor (ER) positive breast cancers. However, some patients acquire resistance over prolonged treatment. The biological mechanism still awaits better understanding. Previously a number of gene expression profiling studies identified gene profiles that could predict cancer prognosis or characterize tamoxifen responsiveness, but there is little concordance between the genes identified. This may be due to pre-mRNA alternatively spliced variants that are not discriminated in conventional microarrays.
To identify the alternatively spliced variants that contribute to tamoxifen resistance, SpliceArray™ profiling (ExonHit Therapeutics, Inc.) on 417 breast cancer related genes was performed in a panel of breast cancer cell lines. Splice variants that were differentially expressed between parental tamoxifen-sensitive (TamS) and derived tamoxifen-resistant (TamR) cell lines were identified and validated by real time-quantitative PCR.
Splice variant BQ323636.1 of NCOR2 (Nuclear receptor co-repressor 2) was successfully validated in cell lines and clinical samples. In presence of tamoxifen, the mRNA expression level ratio of variant (BQ323636.1) versus wild type form (NM_006312.2) was significantly higher in derived TamR cell line AK47 compared to its parental TamS cell line ZR75−1. In 26 Chinese breast cancer patient RNA samples, the ratio positively correlated with metastasis.
NCOR2 is a component of the histone deacetylase-containing protein complex. It is recruited by tamoxifen to repress the transcription activation activity of ERα. Exon 11 skip in BQ323636.1 variant results in early termination of the protein product. Only the first repression domain at the N-terminal is retained while 3 repression domains and 2 nuclear receptor-interacting domains are lost, indicating its disability of binding to nuclear receptors. Thus, this variant may inhibit the repression on ERα transcription activation by competing with its wild type form for interacting with other protein partners in the HDAC complex. This may hinder the recruitment of HDAC complex to the target gene promoter and suppress the antagonist effect of tamoxifen, leading to tamoxifen resistance. Ongoing functional studies are being performed to confirm this possible mechanism, which may serve as a potential therapeutic target to overcome acquired tamoxifen resistance in breast cancer.

238 The effects of proanthocyanidins on cardiotoxic and antitumour activity of doxorubicin

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Background: Mechanisms of proanthocyanidin (PRO) activity are primarily associated with their antioxidative effects. As direct antioxidative action cannot explain positive effects of PRO in prevention of cancer and heart damage, we used different *in vivo* and *in vitro* models and combination of doxorubicin (DOX) and PRO to find out whether and how low doses of PRO could modulate DOX antitumour activity and achieve cardioprotection after DOX treatment.
Material and Methods: PROs were extracted from grape seeds by ethlyacetate and water. Ehrlich ascitic and solid tumours were induced in Hann:NMRI mice. Free radical scavenging activity of PRO was determined by electron spin resonance (ESR) spectrometer. NADPH:cytochrome P450