by Akt and PKA in *in vitro* kinase assays. Substitution of this serine with glutamic acid (S to E) greatly increases estrogen receptor (ER) dependent gene transcriptional activity, while substitution with alanine (A) (S to A) greatly reduced AIB1 coactivity of ER and androgen receptor dependent gene transcription, but not progesterone receptor dependent gene transcription in Hela cells. The S to A mutation in AIB1 doesn't change its cellular distribution with or without estrogen treatment, as examined by fluorescence microscopy. This specific phosphorylation, however, may mediate p300's enhancement of ER-dependent transcription, since the S to A mutation in AIB1 significantly attenuated this activity of p300. Moreover, the specific phosphorylation may have a role in the tamoxifen's agnostic activity, since the S to A mutation partially diminishes tamoxifen-induced ER-dependent gene transcriptional activity, as compared with tamoxifen-induced ER-dependent gene transcriptional activity in the presence of wild type AIB1.
Taken together, the serine phosphorylation site we have identified in AIB1 is critical to AIB1 functions and may serve as a link between the nuclear receptor pathway and other important signaling pathways such as the PI-3-kinase and cAMP pathways. Our data provide a mechanistic explanation for our previous clinical observation that high levels of *both* HER2 and high AIB1 are required for tamoxifen resistance, and serve as a molecular basis for strategy to block signaling from HER2 overexpression to AIB1.

659 POSTER

ER-beta expression in rhabdomyosarcoma cells is essential for activation of caspase mediated apoptosis after treatment with tamoxifen

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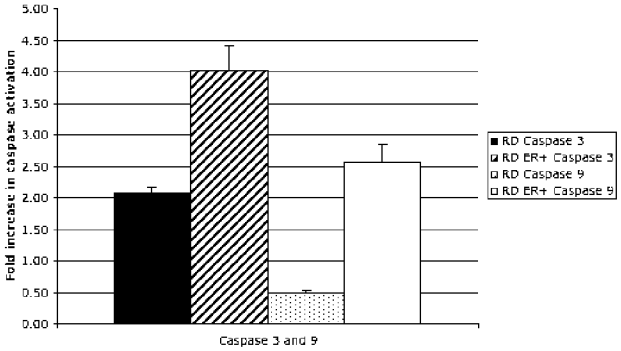
Introduction: Tamoxifen (TAM), a selective estrogen receptor modulator, has a significant effect on the recurrence and survival rates of patients with breast cancer. TAM has also been shown to induce responses in desmoid tumors and aggressive fibromatosis in children. We have previously shown that there is a correlation between the endogenous expression of estrogen receptor beta (ER-beta) in rhabdomyosarcoma (RMS) cells and sensitivity to treatment with TAM. In this study we demonstrate that the sensitivity to TAM is significantly increased by expression of ER-beta in ER-beta negative RMS cells, and that the increased cell-death is caspase-mediated.



Caspase activation 12 hours after treatment with 7.5 uM tamoxifen. The bars show fold increase and standard deviation for caspase activation compared with an untreated control. For both caspase 3 and caspase 9 the difference between RD and RD ER+ was statistically significant ($p<0.002$ and $p<0.001$).

Methods: RD, an ER-beta negative embryonal RMS cell-line, was cultured until 50% confluent, then transfected using a psg5-ER-Beta transfection vector. TAM was added 20 hours after transfection at the following concentrations: 5 μM, 7.5 μM and 10 μM. Successful transfection was verified by western blot analysis using a monoclonal antibody (ER-beta 14C8) to verify high expression levels of ER-beta after transfection. After treatment for 12 hours, caspases 3 and 9 activation were assessed, using a fluorometric assay. After 20 hours, alamar blue viability assay was performed. All experiments were done in triplicate. For statistical analysis, Student's t-test was applied to compare two groups and a p-value of less than 0.05 was considered significant. MCF7, a breast cancer cell line, was used as positive control, and RD transfected with empty psg5 vector served as a negative control.
Results: No detectable expression of constitutive ER-beta was seen in RD. However, western blot analysis showed strong expression of ER-beta in RD cells transfected with ER-beta psg5. The sensitivity to TAM was increased significantly in RD ER-beta expressing cells (RD ER+) compared with RD ER-beta negative (RD) cells for TAM concentrations of 5 μM (92.0% vs 111.8%, $p<0.0001$), 7.5 μM (66.9% vs 89.8%, $p<0.0001$) and 10 μM (29.4% vs 60.9%, $p<0.0001$). Fold increase in caspase 3 activation was significantly higher in RD ER+ compared with RD for TAM concentrations of 5 μM TAM (1.5 vs 0.92, $p<0.02$) and 7.5 μM (4.0 vs 2.1, $p<0.002$) (figure 1). Caspase 9 activation was also significantly higher in RD ER+ as compared with RD for 5 μM TAM (1.2 vs 0.5, $p<0.001$) and 7.5 μM (2.6 vs 0.5, $p<0.001$) (figure 1).
Conclusion: Our findings suggest a biologic mechanism for caspase mediated apoptosis after Tamoxifen treatment, that is dependent on ER-beta expression. These observations provide a basis for development of further studies to examine the in vivo effects of TAM in RMS, and to incorporate its use in clinical trials in the treatment of rhabdomyosarcoma.

660 POSTER

Fulvestrant does not markedly inhibit human cytochrome p450 isozymes: results from in vitro studies

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Background: Fulvestrant (Faslodex®) is an oestrogen receptor (ER) antagonist with no agonist effects that is indicated for the treatment of postmenopausal women with ER-positive breast cancer who have progressed following prior antioestrogen therapy. Patients in this setting are often receiving polypharmacy for comorbid conditions, which increases the likelihood of adverse drug-drug interactions. Here we present a preclinical study designed to investigate the potential of fulvestrant to inhibit the metabolism of model substrates of the cytochrome P450 isozymes CYP 1A2, 3A4, 2A6, 2C8, 2C9, 2C19, 2D6, and 2E1.
Material and Methods: Human hepatic microsomal protein preparations were incubated with substrates of cytochrome P450 isozymes 1A2 (phenacetin), 3A4 (testosterone), 2A6 (coumarin), 2C8 (paclitaxel), 2C9 (tolbutamide), 2C19 (mephenytoin), 2D6 (dextromethorphan), and 2E1 (chloroxazone), at approximate K_m concentrations for each enzyme and fulvestrant (0.003 to 10 $\mu g \cdot mL^{-1}$). P450 enzyme activities were determined by spectrophotometry (coumarin 7-hydroxylase), HPLC with on-line radiochemical detection (paclitaxel 6α-hydroxylase), HPLC with UV detection (chloroxazone 6-hydroxylase; phenacetin O-deethylase; tolbutamide 4′-hydroxylase; S-mephenytoin 4-hydroxylase), or HPLC-fluorimetric assay (dextromethorphan O-demethylase). Enzyme activities were compared with control samples in which fulvestrant had been replaced with water. The effect of pre-incubation with a NADPH-regenerating system on the inhibitory potential of fulvestrant was also assessed (2A6, 2C8 and 2E1 incubations). Selective inhibitors of the various CYP isozymes were used as controls in this model system.
Results: Fulvestrant did not markedly inhibit the activity of any of the human P450 isozymes (<20% inhibition at the highest concentrations of fulvestrant) included in this study. Pre-incubation with NADPH had no effect on the inhibitory potential of fulvestrant, while the degree of inhibition observed with the control inhibitors was comparable to that observed in previous studies.
Conclusions: *In vitro* data suggest that fulvestrant is unlikely to cause clinically significant reversible inhibition or mechanism-based inactivation of CYP isozymes 1A2, 3A4, 2A6, 2C8, 2C9, 2C19, 2D6, and 2E1. Fulvestrant would not therefore be expected to cause significant drug interactions through P450-mediated metabolism of co-administered agents

661 POSTER

An androgen-receptor (AR)-negative human prostate cancer xenograft induces mouse-derived osteogenesis

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Background: The burden of prostate cancer (PC) bone metastases (BM) correlates with survival and a deeper understanding of the pathobiology may offer therapeutic opportunities. PC BM are typically osteoblastic but experimental models rarely recapitulate this phenotype.
Goals: To study malignant epithelial-osteoblast interactions that may be critical determinants of the pathophysiology of PC progression we sought to develop a representative model of osteoblastic bone metastases.
Methods: A SCID mouse xenograft was generated subcutaneously from an exophytic osteoblastic bone metastasis from a patient with androgen-independent PC. Tumors grew progressively and were passaged

serially in bone and subcutaneous sites. Orthotopic bone implants and subcutaneous xenografts were characterized by radiology, histopathology and immunohistochemistry (IHC). Fluorescence in-situ hybridization (FISH) using mouse Y chromosome paint probe was performed to confirm the origin of subcutaneous osteogenesis.
Results: X-ray analysis of orthotopic bone tumors demonstrated osteoblastic lesions. Sera from tumor bearing mice were negative for Prostate Specific Antigen (PSA). Histology of orthotopic bone implants demonstrated a malignant epithelial proliferation associated with new bone formation typical of osteoblastic bone metastases of PC. Additionally, histology of subcutaneous lesions exhibited evidence of heterotopic bone formation. Western Blot analysis and IHC for AR were negative in multiple lesions analyzed. FISH confirmed mouse-derived osteogenesis in subcutaneous sites. IHC demonstrated staining for multiple growth factors including bone matrix proteins and platelet-derived growth factor receptor.
Conclusions: An experimental model of AR-negative osteoblastic PC has been developed which induces orthotopic and heterotopic bone formation via paracrine mechanisms. AR and PSA-independent mechanisms may contribute significantly to the progression of prostate cancer in bone. This model may help define these mechanisms, which in turn may define alternative therapy targets.