1047 POSTER

Annexin-A1 Mediates Chemosensitivity to PPAR-gamma Ligands in Mammary Carcinoma: a Novel Biomarker for Effective Tailoring of Patients to PPAR-gamma Ligand Therapy

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Background: Annexin-A1 (ANXA1), a calcium-dependent phospholipids-binding protein, has been implicated in the development of breast tumorigenesis. However, evidence on the exact role of ANXA1 in breast cancer is limited. Peroxisome proliferator-activated receptor gamma (PPARg) offers a molecular target for drugs aimed to treat type II diabetes mellitus, while its therapeutic potency against cancer disease, including breast cancer is well established. Despite achievements on improved current clinical therapies and medical treatments on breast cancers have been continuously reported for the past decade, a good biomarker is needed for better screening and selection of cohort of patients for breast cancer therapy in future clinical trials.
Material and Methods: Crystal violet staining and AnnexinV-PI staining were used to measure percentage of cell death. Luciferase reporter assay were used to measure promoter activities of PPARg and ANXA1, and NF-kB transcription activity. Protein expression and mRNA expression levels were measured by Western blotting and real time PCR respectively.
Results: A screen on a panel of breast cancer cell lines reveals ANXA1 is highly expressed in invasive breast cancer cell lines such as MDA-MB-231 and BT549, while non-invasive cell lines such as MCF-7 and T47D show much lower expression of ANXA1. This corroborates our published study where high ANXA1 expression was observed in breast cancer patients with high grade tumour while patients with low grade tumour express low ANXA1 protein. Exposure of breast cancer cells MDA-MB-231 and BT549 to PPARg ligands resulted in upregulation of ANXA1 transcription as well as protein expression with corresponding inhibition in cells' viability through a PPARg-NFkB-ANXA1 axis. Suppression of ANXA1 levels by small-interfering RNA in these cells conferred resistance to PPARg ligand treatment. On the other hand, expression of ANXA1 in MCF-7 and T47D cells and viability remained unchanged with the same dose treatment. Interestingly, sensitivity to PPARg ligands could be achieved in MCF-7 and T47D cells by forced over-expression of ANXA1. Further, MDA-MB-231 xenograft model in nude mice treated with PPARg ligands, showed a significant reduction in tumour size, and tumour tissues stained by immunohistochemistry showed increased ANXA1 protein levels.
Conclusion: Despite the numerous preclinical data supporting for the use of PPARg ligands as anti-cancer drugs, none of the clinical trials have been successful. Herein, we propose that perhaps we could use expression levels of ANXA1 as a biomarker to better select cohort of patients for PPARg ligand therapy in clinical trials.

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Ligand Activation of PPAR Gamma Enhances Cytotoxicity of Chemotherapeutic Drugs in Breast Cancer Cells: the Mechanism Involving Tumour-specific Suppression of Mitochondrial MnSOD in Vitro and in Vivo

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Background: Ligand induced peroxisome proliferator-activated receptor gamma (PPARγ) activation has been reported to inhibit the proliferation of malignant cells, possibly through reactive oxygen species (ROS) production. An increasing number of studies have demonstrated that not only do various therapeutic approaches depend on ROS, but that further elevation of cellular ROS can indeed kill cancer cells more effectively. Manganese superoxide dismutase (MnSOD) is one of the major antioxidant enzymes overexpressed in many cancers and could regulate ROS-mediated cell death induced by PPARγ activation.
Materials and Methods: NoShift Transcription factor assay was used to measure binding affinities of PPARγ to MnSOD promoter. Luciferase reporter assay was used to measure PPRE promoter activity. Real-time PCR and western blot analysis were used to determine MnSOD mRNA and protein levels. MitoSOX Red assay was used to measure mitochondrial superoxide levels. Cell viability assays were used to determine sensitivity of cells upon drug treatment. Immunohistochemistry was used to stain for MnSOD levels in mouse and human tumour tissues.
Results: We report the identification of PPARγ response elements within the human MnSOD promoter region and that activation by low "sensitizing doses" of PPARγ agonists led to significant downregulation of MnSOD mRNA and protein levels. Also, a corresponding receptor-dependent increase in intracellular superoxide production in breast cancer cells was observed. Importantly, normal breast cells were completely refractory to this effect due to low levels of PPARγ protein. Suppression of MnSOD levels by small-interfering RNA or PPARγ agonists in breast cancer cells reduced their colonogenic ability and enhanced chemo-sensitivity to ROS-promoting drugs such as docetaxel and doxorubicin, which could be abrogated by MnSOD overexpression. Furthermore, MDA-MB-231 xenograft model in nude mice treated with PPARγ ligands showed a significant reduction in tumour size, and tumour tissues stained by immunohistochemistry showed a decrease in MnSOD protein levels. Finally, histopathologic analysis of breast cancer biopsies obtained from patients with type II diabetes treated with synthetic PPARg agonists showed significant repression of MnSOD in the tumour tissues.
Conclusion: Together, our data not only identifies MnSOD as a novel target of PPARγ but also provides a molecular mechanism for ROS-manipulation therapy in the clinic through the intelligent use of PPARγ ligands in combination with ROS-promoting drugs such as doxorubin or docetaxel.

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Increased Amplification of HER2 in a Cell Line Model of Acquired Lapatinib Resistance

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Background: Lapatinib is a reversible small molecule inhibitor of HER2 and EGFR, which is approved for the treatment of trastuzumab refractory metastatic breast cancer. However, resistance to lapatinib is a significant clinical problem and to date the mechanisms of acquired lapatinib resistance remain largely unknown. We have developed an *in vitro* model of acquired lapatinib resistance by conditioning the HER2 positive, lapatinib sensitive HCC1954 cells with lapatinib for 6 months.
Materials and Methods: HCC1954 cells were conditioned with 1 mM lapatinib for 6 months and the resulting cell line is referred to as HCC1954-L. Proliferation assays were performed across a range of lapatinib concentrations and measured by acid phosphatase assays. Doubling times were measured over 7 days using Guava viacounts (Millipore). Array CGH analysis was performed; briefly DNA was extracted from cell pellets, fragmented and labeled with Cy5 and Cy3 dyes, hybridized to arrays, scanned by a microarray scanner and analyzed using Agilent analytics software. Western blotting was performed with a HER2 specific antibody (Calbiochem) on parental and resistant cell protein lysates.
Results: HCC1954-L cells are resistant to lapatinib (IC_{50} 2.574±0.251 mM) compared to parental cells (IC_{50} 0.424±0.018 mM; $p = 0.004$). The resistant phenotype is stable in the absence of lapatinib for 3 months. Lapatinib treatment significantly increases the doubling time of the parental cells (83.0±3.6 compared to 29.8±1.0 hours; $p = 0.0007$). Lapatinib does not significantly affect the doubling time of HCC1954-L cells (57.2±7.1 compared to 48.5±2.6 hours; $p = 0.2$).
Array CGH (comparitive genomic hybridization) analysis revealed a significant amplification (1.64 fold; $p < 0.0001$) of the HER2 gene in HCC1954-L cells compared to the parental cells. Increased HER2 protein expression was observed in the HCC1954-L cells compared to the parental HCC1954 cells, by western blot analysis.
Conclusions: HCC1954-L cells represent a stable *in vitro* model of acquired lapatinib resistance. CGH analysis revealed further amplification of HER2 at the gene level and an increase in total HER2 protein expression was confirmed by western blotting. Increased expression of HER2 represents a possible mechanism of acquired resistance to lapatinib in this cell line.

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Copy-neutral Loss of Heterozygosity at the P53 Locus in Esophageal Squamous Cell Carcinomas Associated With p53 Mutations

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Purpose: The elucidation of the mechanisms causing loss of heterozygosity (LOH) in esophageal squamous cell carcinoma (ESCC) will give us further understanding of its carcinogenesis and will also have preventive, diagnostic, and therapeutic implications for this aggressive disease. Loss of tumour suppressor gene function is generally thought to occur in two steps, the first being mutation in one allele followed by the somatic loss or inactivation of the second allele, or LOH. The aim of this study is to