due to the strong family history. Peripheral blood samples were collected from the breast cancer proband and was processed for DNA and RNA extraction. 16-exon CDH1 gene sequencing was performed to exclude the exon 13 mutation reported in a Korean kindred with coexisting ductal breast and gastric cancer. In addition, the entire coding regions and flanking introns of BRCA1/2 were screened for germline mutations using full gene sequencing and Multiplex Ligation-dependent Probe Amplification (MLPA).

Results: Sequencing of the 16-exon CDH1 gene was negative. An Exon 18 duplication causing a frameshift mutation and downstream premature termination codon (8047_8054dup GCAAAAAC, Leu2686GluX10) was revealed, this being a novel BRCA2 mutation not previously reported.

Conclusion: We identified a novel BRCA2 germline mutation in a Korean breast cancer patient with extensive history of non breast cancer. There is limited knowledge of the prevalence, spectrum of germline mutations and also the phenotypic presentations in Asian population. Research on the spectrum of mutations in such diversed ethnic groups has important implications on clinical management.

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Expression and functional analysis of PGRMC1 in breast cancer

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Background: In a proteomic screening approach we identified progesterone receptor membrane component 1 (PGRMC1) as a protein upregulated in frozen ER-negative breast cancer tissue samples making PGRMC1 a potential therapeutic target. PGRMC1 is a 28 kDa protein consisting of 194 amino acids and belongs to the "membrane-associated progesterone receptor" (MAPR) protein family.

Aim: The aim of this project is to characterize the potential biological function of PGRMC1 by structure analysis and generation of different mutant forms.

Material and Methods: Structure analysis was performed (http://scansite.mit.edu/motifscan_id.phtml) under high and medium stringency to identify protein motifs predicted for PGRMC1. Based on these predicted motifs PGRMC1-variants were generated by site-directed mutagenesis and MCF-7 breast cancer cells were stably transfected with the corresponding expression plasmids. Several PGRMC1-specific hybridoma were generated and tested by western blot analysis. Multiple immune fluorescence for PGRMC1, estrogen receptor alpha and the hypoxia marker glucose transporter 1 (Glut1) was applied to label tissue microarrays containing different breast cancer specimen. Additionally, posttranslational modification of PGRMC1 was investigated by phosphatase treatment of tissue lysates. Functional analysis was performed by stimulating transfected MCF-7 cells with a membrane-impermeable progesterone:BSA:fluoresceinisothiocyanate conjugate followed by analysis of cell proliferation by measuring the cellular ATP content.

Results: PGRMC1 contains a cytochrome b5 domain and several protein interaction domains making it a possible signalling adaptor protein. Further, PGRMC1 is posttranslationally modified. Stimulation of PGRMC1 expressing MCF-7 cells resulted in an increased proliferation compared to control cells grown in growth factor and hormone reduced medium. Western blot analysis of hybridoma produced a PGRMC1-specific signal at the predicted molecular weight of 28 kDa which is not detected after preincubation of the hybridoma with recombinant PGRMC1 protein. In immune fluorescence analysis of breast cancer tissue sections myoepithelial cells were highly positive for PGRMC1. Besides that PGRMC1 expression was upregulated in Glut1 positive, hypoxic areas in ductal carcinoma in situ of comedo type.

Conclusion: These data indicate that PGRMC1 is expressed in breast cancer and might functionally play a role in cell proliferation. Further determination of the so far poorly defined role of PGRMC1 in cancer biology could prove to be of great relevance to clinical cancer therapists.

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Peroxisome proliferator-activated receptor-gamma agonist, troglitazone has anticancer effect on breast cancer cells

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Background: It is reported that peroxisome proliferator-activated receptor-gamma (PPAR-γ) has become a potential target for the prevention and treatment of breast cancer. And also, recent studies suggest that PPAR-γ agonist could serve as negative regulators of breast cancer development and progression, but their mechanism is still unknown. The purpose of this study was to evaluate the mechanism that PPAR-γ agonist, troglitazone would induce antiproliferative effect on MCF-7 (ER-positive) and MDA-MB-231 (ER-negative) breast cancer cells.

Methods: Cytostatic/cytotoxic effects of troglitazone were measured with mitochondrial tetrazolium (MTT) assay. The cell cycle distribution and apoptosis induction were evaluated by using the flow cytometry. The expression of apoptosis-related proteins were measured with Western blotting. Detection of apoptosis was carried out using a DNA fragmentation assay based on TUNEL staining. For morphological examination of apoptotic changes, cells were stained with Hoechst 33342.

Results: Troglitazone showed antiproliferative effect on MCF-7 breast cancer cells with tamoxifen, respectively and synergically. Troglitazone and tamoxifen could induce cell cycle G1 arrest and apoptosis of MCF-7 cells, through upregulating or downregulating the expression of apoptosis-related genes. MDA-MB-231 cells exposed to troglitazone showed G1 arrest as well as induction of characteristic morphological changes of apoptosis. Accumulation of cells in G1 was accompanied by an attenuation of retinoblastoma (Rb) protein phosphorylation associated with decreased cyclin dependent kinase (CDK) 2 activity. Troglitazone increased the expression of CDK inhibitor, p21 and p27.

Conclusion: PPAR-γ agonist, troglitazone increase the sensitivity of anti-hormonal therapy in MCF-7 breast cancer cells and inhibits the proliferation of MDA-MB-231 cells. These results suggest that troglitazone has anticancer effect on both ER-positive and negative breast cancer cells.

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Tamoxifen as inhibitor of multidrug resistance mechanism in lung and breast cancer tumours

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Background: Tamoxifen (Tam) is an effective drug in standard therapy of breast cancer as an antiestrogen. At the same time there a lot of other important activities of Tam, one of them – increase chemotherapy efficacy by high doses of Tam. Analyzing the results we have taken notice that in all cases Tam was used in chemotherapy included anticancer drugs associated with multidrug resistance mechanism (MDR) that is determined by energy-dependent ABC-transporters, extruding MDR-drugs out of the cells. Besides it was shown that Tam increases cytotoxicity of MDR-drugs in cell cultures with expression of Pgp. That is why thinking over the reasons of increase chemotherapy efficacy under Tam action we supposed the one of the reasons for that may be Tam inhibition of ABC-transporter(s)' activity.

Material and Methods: Tam influence on intracellular accumulation of MDR-drug doxorubicin (Dox) in tumor cells of surgical biopsy specimens (breast cancer and non-small-cell lung cancer, totally – 22 specimens) was studied by a flowcytometry. MDR-phenotype (Pgp+ and/or MRP+) of the investigated tumors was determined as the change in Dox intracellular accumulation after preincubation of cell suspension with ABC-transporter(s)' inhibitors: verapamil (Ver) and genistein (Gen) – specific inhibitors of Pgp and MRP respectively.

Results:

1. Totally, stimulation of Dox intracellular accumulation after Ver and/or Gen action (Pgp+ and/or MRP+ phenotype) was shown in 70% of investigated tumor specimens.
2. Tam stimulated Dox intracellular accumulation in all tumor specimens with Pgp+ and/or MRP+ phenotype.
3. Significant increase in nuclear fraction and Dox binding to DNA was also revealed in stimulation of Dox intracellular accumulation under Tam action.

Conclusions:

1. Tam is an effective inhibitor of ABC-transporter(s)' functional activity namely Pgp and MRP.
2. Tam inhibition of ABC-transporter(s)' function resulting in increase in Dox intracellular accumulation and nuclear fraction of Dox could at least partly explain efficacy of MDR-drugs in combinations with high doses of Tam.

It means that Tam could be considered not only an effective antiestrogen drug but also an inhibitor of multidrug resistance mechanism increasing efficacy of MDR-drugs in tumors exhibited MDR-phenotype Pgp+ and/or MRP+. According to presented results it could be true at least for lung and breast cancer.

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