Porto, Portugal. DNA extracted from peripheral blood was submitted to Polymerase Chain Reaction (PCR) followed by Restriction Fragment Length Polymorphism (RFLP), in order to identify the *CYP2D6* genotypes. The mean disease free survival time was assessed using Kaplan-Meier methodology and the log rank test.
Results: From the global sample, the *CYP2D6* polymorphism was observable in 79 patients: CYP2D6 homozygotic (wt) was present in 59.5% of all cases, heterozygotic in 36.7% and homozygotic poor metabolizer (pm) in 3.8%. The mean disease free survival time (months) was significantly better in the patients that are carriers of the *CYP2D6* wt genotype (215 vs 46, $p = 0.028$). This was particularly evident in early stages (Stages I and II), with a mean disease free survival time of 247 ± 39 for homozygotic wt genotype carriers and 49 ± 6 for heterozygotic and pm homozygotic genotypes carriers ($p < 0.001$).
Conclusion: Our results suggest a role for *CYP2D6* polymorphisms in the clinical outcome of early onset breast cancer patients. The characterization of the drug metabolising genetic individual profile might lead to an individual chemotherapy approach, which would allow drug dosing on an individual's capacity to respond, thus leading to a more efficient and less toxic treatment.

316 PUBLICATION

Analysis of BRCA1 and BRCA2 mutations in high-risk patients from the Prague-area

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Background: About 5–10% of all breast cancer cases are due to inheritance of a susceptibility allele and a substantial proportion of these are due to germline mutations of the two major highly penetrant cancer susceptibility genes, *BRCA1* and *BRCA2*. The purpose of this study was to estimate the incidence, spectrum and possible clustering of disease phenotypes associated *BRCA1* and *BRCA2* mutations. The analysis was performed in breast/ovarian cancer families and in high-risk patients not selected on the basis of their family history of cancer.
Material and methods: 122 Czech families with recurrent breast and/or ovarian cancer and 69 patients considered to be at high-risk but with no reported family history of cancer were screened for mutations in the *BRCA1/2* genes. The entire coding region of each gene was divided into overlapping fragments with a size range of 880–1569 bp and amplified by the polymerase chain reaction. Mutational analysis was carried out by the protein truncation test and direct DNA sequencing.
Results: Within 191 analyzed individuals, 48 (25.1%) carried a *BRCA1* mutation and 10 (5.2%) a *BRCA2* mutation. One novel truncating mutation was found in *BRCA1* (c.1866 A>T) and two in *BRCA2* (c.4167delC and c.5991dupT). *BRCA1* mutations comprised 14 different alterations. Five recurrent mutations accounted for 81.2% of individuals with detected gene alterations. The *BRCA1* 5382insC detected in 56.2% of mutation positive women was the most prominent gene defect. A total of 8 different mutations were identified in *BRCA2*. The novel c.5763dupT mutation and c.5682C>G, which appeared in two unrelated families each, were the only recurrent alterations of the *BRCA2* gene. Pathogenic mutations were found in 24.0% of breast cancer families and in 62.8% of families with the occurrence of both breast and ovarian cancer. In addition, deleterious mutations were detected in 10.0% of women with early-onset breast cancer. A total of 4 hereditary mutations in *BRCA1* were identified among 17 (23.5%) women with a medullary breast carcinoma selected for examination regardless of the family history.
Conclusions: Mutational analysis of *BRCA1/2* genes characterized the spectrum of gene alterations in Czech population and demonstrated the dominant role of the *BRCA1* c.5382insC allele, which accounted for more than 46% of all identified gene alterations.
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317 PUBLICATION

Epidermal growth factor receptor levels in progesterone receptor positive breast tumors

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Introduction: Some breast tumors express epidermal growth factor receptor (EGFR) in different concentrations, and it has been related with poor prognosis. Higher levels of EGFR are related with hormonal receptors negative status. Our purpose is observing the behaviour of EGFR depending on different cut-off values of progesterone receptor (PR) positive breast cancer tissues.
Patients and methods: 472 patients aged between 27–88 years old were analyzed. 268 breast tissues with infiltrative ductal carcinoma (IDC) were used to measure progesterone receptor and EGFR. Hormonal receptor was determined with quantitative enzymatic immunoassay. EGFR was measured with radioligand assay. Statistic analysis was performed with Mann-Whitney U test.
Results: In the following PR cut-off points, the results were:
- >1 fmol/mg cytosol protein: median (p50) EGFR levels in PR positive tumors were 3.9 fmol/mg cytosol protein vs 4.25 in PR negative tumors (non signif.)
- >5 fmol/mg cytosol protein: median EGFR levels in PR positive tumors were 3.7 vs 4.75 in PR negative tumors ($p < 0.001$).
- >10 fmol/mg cytosol protein: median EGFR levels in PR positive tumors were 3.65 vs 4.45 in PR negative tumors ($p < 0.05$).
- >15 fmol/mg cytosol protein: median EGFR levels in PR positive tumors were 3.6 vs 4.5 in PR negative tumors ($p < 0.05$).
- >20 fmol/mg cytosol protein: median EGFR levels in PR positive tumors were 3.55 vs 4.45 in PR negative tumors ($p < 0.05$).

Conclusion: Higher values of EGFR were measured in PR negative samples of IDC of the breast using five different cut-off points of positivity, starting in 5 fmol/mg cytosol protein. We can conclude that EGFR levels show inverse relation with hormone dependent infiltrative ductal carcinoma of the breast.

318 PUBLICATION

Antisense chemoradioimmunotherapy inhibit the endothelin axis with subsequent induction of type I, type II PCD and metastatization in advanced breast cancer characterised by hypermethylated oncosuppressor promoter CpG islands and overexpression of oncogenes

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Advanced breast cancer is resistant to almost all cytotoxic drugs and radiation making it one of the most aggressive malignancies in humans with the worst mortality. The failure of tumour cells to undergo apoptosis cause resistance to chemoradiological therapies due to overexpression of oncogenes and transcriptionally repressed apoptotic tumour suppressor genes due to aberrant methylation (CIMP+). Also, overexpression of endothelins enhances tumour proliferation.
We obtain tumour cells from a patient with metastatic breast cancer MS-PCR detected methylation of tumour suppressor genes p53, p16, RASSF1A, RAR-b2, BRCA2, PTEN, E-cadherin, hMLH1, ESR1, CDH1, TRbeta1, GSTP1 and CCND2. Quantitative IHC, WB, SB and PCR exhibited overexpression of COX-2, PGE2, bcl-2, ET-A, Raf-1, cdc25c, c-fos, c-myc, c-jun, EGFR and VEGF. We treated the tumour cells with antiET-A scFv attached onto high energy radioisotopes, vinorelbine tartrate and 21 nucleotide double standed siRNA segment generated against DNMT1. Post-treatment, we detected re-expression of oncosuppressor genes after inhibition of DNMT1mRNA. Downregulation of paracrine/autocrine factor ET-A due to targeted scFv inhibited the endothelin induced signal transduction pathways by blocking binding of ET-1 to the ET-AR in the plasma membrane.
This blocked the signal transduction pathway through G9 causing inactivation of PLC, PTKs such as FAK and RAS blocking the RAF/MEK/MAPK pathway. Inhibition of ET-1/ETAR caused downregulation of ILK, IOGAP1, a2, b3 and b1 integrins, N-cadherin, COX2, PGE2, VEGF and upregulation of connexin, E-cadherin and b-catenin. This inhibited intracellular Ca++, PKC, MAPK, p42/44MAPK kinase and p38 MAPK blocking transcription of EGFR, c-fos, c-myc and c-jun leading to inhibition of cell growth and mitogenesis. It also inhibited PIK3-mediated AKT activation. Vinorelbine caused inactivation of bcl-2, Raf-1 and cdc25c by phosphorylation. We detected upregulation of p21Waf1, p27Kip, E-cadherin and Bak. The high energy radioisotopes induced DNA double strand breaks in tumour cells arresting synergistically with MT depolymerizing VRL their growth at the G2/M transition according to flow cytometry. We detected externalisation of PS, depolarization of mitochondrial transmembrane potential, activation of caspase 3.9, bax and DNA fragmentation. TEM exhibited irreversible D2 apoptotic signs forming apoptotic bodies indicating typeI PCD after chromatin condensation and nuclear fragmentation. Overexpression of Beclin-1, PTEN, p70, DAPK and BNIP3 induced ceramide mediated autophagic cell death termed as typeII PCD where LC3 is localised in autophagosome membranes. BrdU and MTT exhibited inhibition of DNA synthesis and metabolic activity of treated tumour cells compared to untreated controls.
We have achieved to induce type I and type II PCD leading to eradication of advanced breast Ca cells which is correlated with inhibition