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

of mitogenesis, invasion, neoangiogenesis and metastatization with combined chemoradioimmunotherapy after circumvention of chemo- and radioresistant mechanisms such as hypermethylation of oncosuppressor genes, overexpression of oncogenes and inhibition of endothelin induced signal transduction pathways

319 PUBLICATION

p27 Deregulation as a prognostic marker in inflammatory breast cancer

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Introduction: Deregulation of p27 manifested by low protein cellular concentration has been shown to be an independent poor prognostic factor in breast cancer. The objective of this study was to evaluate whether the deregulation of p27 is a prognostic factor in patients with inflammatory breast carcinoma (IBC).
Patients and methods: Fifty-eight IBC patients were treated between January 1994 and July 2002 in clinical trials. Thirty-eight patients with baseline biopsy specimens and adequate follow-up data were included in this study. Patients were treated with preoperative chemotherapy with FAC (5-fluorouracil 500 mg/m^2, doxorubicin 50 mg/m^2 and cyclophosphamide 500 mg/m^2) (N = 13, 34%), or FAC followed by a taxane (paclitaxel 175–250 mg/m^2, or docetaxel 100 mg/m^2 every 21 days) (N = 25, 66%). All patients obtained objective clinical responses and underwent mastectomies. Eight patients received adjuvant paclitaxel; all patients received radiotherapy and hormonal treatment when indicated.
Expression level of p27 was evaluated by standard indirect immunoperoxidase procedure (nuclear staining). Tumors with p27 staining in less than 50% of the neoplastic cells were called p27-deregulated; tumors with p27 staining in equal or more than 50% of the neoplastic cells were called p27-normal or not-deregulated.
Results: Median age at diagnosis was 49 years, (21–73 years). Thirty-two patients (84%) had p27-deregulated tumors and 6 patients (17%) had p27-normal tumors. Six patients (17%) achieved a pathologic complete response (pCR). At a median follow-up of 43 months, 25 recurrences (66%) and 27 deaths (71%) had occurred. Patients with p27-deregulated tumors had fewer pCR (deregulated: 3/32–9%; not-deregulated 3/6–50%; $P = 0.03$) and had lower 4-year RFS (23% vs. 83%, $P = 0.03$) and OS rates, (36% vs. 83%, $P = 0.01$). Due to the small number of patients, the multivariate analysis failed to show any independent predictors of RFS or OS.
Conclusions: This retrospective analysis demonstrates that p27 deregulation manifested by low protein cellular concentration may represent an adverse prognostic marker in IBC and may provide a valuable tool for selecting treatment for this aggressive disease.

320 PUBLICATION

High frequency of population-specific mutations of BRCA genes among breast cancer patients in the Czech Republic

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Introduction: Breast cancer is the most frequent malignancy in women in the Czech republic. Search for high-risk patients is in the focus of oncologists and gynecologists. A part of breast carcinomas (5–10%) arises due to hereditary disposition. The main cancer susceptibility genes responding for more than 80% of these cases are the *BRCA1* and *BRCA2* genes. Several hundred different mutations were detected in these genes to date. In certain populations, there is limited pool of mutations – one or several few mutation comprise majority of detected alterations. F.e. in Ashkenazi Jews, 1% of women are carrier one of the three mutations (185delAG, C61G or 5382insC), in Island population almost all the hereditary breast cancer cases are due to one *BRCA2* mutation (999del5). In population of the Czech Republic, three inactivating *BRCA1* mutations (c.5385dupC – 40%, c.3819_3823delGTAAA – 10%, c.300T>G – 10%) comprise 60% of all the alteration detected in both *BRCA* genes (Pohlreich et al.: Med Princ Pract 2003; Foretova et al.: Hum Mutat 2004).
Methods: We analyzed two population-specific mutations – c.5385dupC and c.3819_3823delGTAAA – in both a group of unselected breast cancer patients and group of unaffected age-matched women. The analysis of c.5385dupC was carried out by PCR-mediated site-specific mutagenesis: a mismatch nucleotide was by modified primer introduced during PCR into amplified fragment near the site of mutation. This mismatch together with non-mutated sequence made up a recognition site for restriction endonuclease Dde I. The wild-type (non-mutated allele was restricted, while the mutated allele remained uncleaved. In the detection of c.3819_3823delGTAAA mutation, we took advantage of spontaneous generation of heteroduplexes during PCR with low annealing temperature and the PCR products were simply analyzed by agarose gel electrophoresis. Suspected mutations were confirmed by direct sequencing of appropriate PCR products.
Results and conclusions: The c.5385dupC and the c.3819_3823delGTAAA mutations were analyzed in 592 patients and 474 controls. Seven mutations were detected in a group of patients (1.18%) and no mutation was detected in controls. None of positively tested patients fits the selection criteria for complete BRCA1/2 genetic analysis. Based on these and previously reported data, we can approximate the frequency of *BRCA1/2* mutations in women affected with breast cancer to be one in fifty cases (2%). At this time, the analysis proceeds and large number of patients and other population-specific mutations (c.300T>G in *BRCA1* and c.5991dupT in *BRCA2*) are screened. Although the complete mutational analysis of both *BRCA1* and *BRCA2* genes is very difficult and time-consuming approach and should be available only for risk patients indicated according consensus criteria, targeted analysis of population-specific mutation is extremely fast, simple and cheap and can be used for population screening (in accord with the frequency of mutation occurrence) of breast (and perhaps ovarian) cancer patients.

321 PUBLICATION

Evaluation of blood based detection methods for NMP66, a breast cancer associated nuclear matrix protein complex

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Background: We investigated the performance of two methods of detecting NMP66, a nuclear matrix protein complex, in discriminating between serum from women with and without breast cancer in a cohort of 298 prospectively collected samples.
Materials and Methods: The NMP66 nuclear matrix protein complex has been demonstrated to be present in the sera of breast cancer patients and absent from the sera of women with no evidence of breast disease (Exp. Rev. Mol. Diag. 2:23, 2002). A signature protein of the complex was first isolated on a nickel affinity surface and identified by time of flight mass spectrometry using a SELDI-TOF instrument (Ciphergen Corp., Inc.). Subsequently, a prototype two-site colorimetric sandwich immunoassay for the identified protein was developed and a qualitative reverse transcriptase-polymerase chain reaction (RT-PCR) assay was constructed using probes specific for unique nucleic acid sequences found within the NMP66 complex (Development of a Blood Based Immunoassay to Detect the NMP66 Breast Cancer Associated Nuclear Matrix Protein, 4th European Conference: Perspectives in Breast Cancer, Seville, Spain 2004). The current investigation evaluated the performance of these methods in discriminating between samples from patients with and without breast cancer. Pre-biopsy blood samples were prospectively collected from 208 women who had suspicious mammograms and/or palpable breast lesions. Subsequent pathological examination determined that 55 had cancer (15 DCIS, 6 LCIS, 3 invasive lobular, 26 T1, 3 T2, 1 T3, 1 T4) and 153 had benign breast conditions. Fifteen samples collected from women with metastatic breast cancer and 75 from women who had no evidence of disease on two sequential mammograms in two years were added to the cohort. All samples (N = 298) were blinded by a third party and tested using both methods for detection of NMP66.
Results: The qualitative RT-PCR correctly identified over 70% of the cancer samples, and the colorimetric immunoassay demonstrated good differentiation of noncancer samples, ruling out 80% of those from patients with benign breast disease or no evidence of disease. There was a 67% greater likelihood of cancer being present when the colorimetric test was positive compared to cancer being present when the test was negative among patients who had clinical or biopsy confirmation of breast disease (RR 1.67). This effect was even greater for postmenopausal women, among whom there was a 75% likelihood of cancer being present upon pathological confirmation when the immunoassay result was positive (RR 1.75).
Conclusions: These preliminary results suggest that measurement of the NMP66 protein and associated complexes or products could provide utility in diagnosis of patients with a cost-effective blood-based method. Additional investigations and data are being compiled.