processed immediately after collection to avoid interference of the *in vivo* gene expression signature with ex vivo stress responses. An alternative is the use of an integrated system for collection, stabilization, and purification of intracellular RNA from whole blood like PAXgene (Qiagen) or Tempus Blood RNA tubes (Applied Biosystems). RNA profiles will be stabilised for up to 5 days at room temperature.

300 POSTER

Importance of XRCC1 Arg399Gln polymorphism in the development of breast carcinoma in women with and without breast cancer family history

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Background: Breast cancer is the neoplasia with higher incidence and mortality in women all over the world. Many environmental factors have been associated with risk of breast cancer development, such as radiation, diet and endogenous and exogenous estrogens. Several studies have reported that the genes involved in DNA repair and maintenance of genome integrity are implicated in protecting against mutations that lead to cancer. Epidemiologic evidence has shown that inheritance of genetic variants (polymorphism) at one or more loci results in reduced DNA repair capacity and increased cancer risk. Base excision repair (BER) is a crucial pathway in the maintenance of genome stability. Variants of several DNA repair genes, including *XRCC1* gene, have been described, but the influence of these genetic variants in repair phenotype and cancer risk remains unclarified.
Aim: The purpose of this study was to evaluate the role of *XRCC1 Arg399Gln* polymorphism as genetic susceptibility markers to familiar and sporadic breast cancer.
Materials and methods: We have used a case-control study. We analysed 630 DNA samples from Portuguese individuals: 71 breast cancer patients with family history (FH) of breast cancer, 219 patients without FH and 340 control subjects, for *XRCC1 Arg399Gln* polymorphism using PCR-RFLP.
Results: We found *Arg/Arg* genotype in 33.8% breast cancer patients with FH, in 43.8% of patients without FH and 34.3% of healthy women. We observed statistically significant differences in *Arg/Arg* genotype of *XRCC1 Arg399Gln* polymorphism between breast cancer patients without FH and control group ($p = 0.025$; OR = 1.49, 95%CI: 1.03–2.14). Furthermore, we found that *Arg/Arg* genotype is more frequent in breast cancer patients without FH (43.8%) than in patients with FH (34.3%).
Conclusions: These preliminary results, in the Portuguese population, show a higher frequency of the *Arg/Arg* genotype of *XRCC1 Arg399Gln* polymorphism in patients without FH of breast cancer than in patients with FH and control groups, suggesting this genotype in women with no FH of breast carcinoma as a susceptibility factor to the breast carcinoma development.

301 POSTER

Very high frequency of BRCA1 5382insC founder mutation in Russian "hereditary-like" breast cancers

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Background: BRCA1 5382insC is a rare founder mutation described in Ashkenazi Jews. Some studies indicate that it may play a certain role in breast cancer (BC) incidence in Eastern Europe.
Material and methods: We analyzed the impact of BRCA1 5382insC founder mutation in BC predisposition in St.-Petersburg, Russia. In order to enhance the design of the study, we recruited a significant number of patients with "extreme" level of BC susceptibility (bilateral breast cancer (biBC) patients and/or cases with affected first-degree relative(s) and/or young BC cases) as well as an additional "cancer tolerant" control group consisting of elderly tumor-free women. BRCA1 5382insC allele was detected by allele-specific PCR.
Results: The BRCA1 5382insC carriers constituted as many as 16/184 (9%) familial and/or early-onset ($\leqslant 40$ years) BC cases and 15/144 (10%) biBC patients. The remaining BC cases, i.e. those selected against the early onset, bilaterality, and history of the disease in first-degree relative(s), showed the 5382insC mutation in 18/709 patients (2.5%). Strikingly, the 5382insC variant was not observed in any of 478 middle-aged healthy female donors or 350 elderly ($\geqslant 75$ years) non-affected women.
Conclusions: When taken together with the literature data, several aspects of this study deserve a critical discussion. 1) Unexpectedly for such a numerous nation as Russians, BRCA1 5382insC founder mutation constitute an indeed significant proportion of "hereditary-like" BC cases; 2) Since high BRCA1 5382insC occurrence was also repeatedly observed in BC-affected subjects from other Slavic countries, one may suspect that an initial allocation of this variant to the Jewish ancestry could have been wrong; 3) If the estimates found in this study are coupled together with our results on the frequency of CHEK2 1100delC (6%) and NBS1 657del5 (1%) variants in "hereditary-like" BC, 2 conclusions can be made: a) 3 simple PCR tests may reveal the genetic cause in 1 out of 6 familial and/or bilateral and/or early-onset BC in Russia; b) "comparison of extremes" approach provides a straightforward tool for the disease association analysis of rare genetic variants.

302 POSTER

Chromosome 22 array-CGH profiling of breast cancer reveals tumor heterogeneity and 340 kb shared region of loss with ovarian cancer

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Breast cancer is the leading cause of death among women in developed countries. Loss of chromosome 22q has been previously established as a common event in breast malignancy, with a frequency of up to 66%. However, despite the number of studies (LOH, metaphase CGH), the identity of putative gene(s) on 22q involved in initiation and/or progression of this tumor remains unknown.
To address this issue, we have performed gene copy number profiling in a set of various stage breast cancers, corresponding surrounding healthy tissue and peripheral blood lymphocytes using a tiling-path chromosome 22 genomic microarray, with an average resolution of 75 kb. The major aberration observed was heterozygous interstitial deletions of various sizes in the telomeric part of 22q. The extent of these deletions varied from 340 kb to 12 Mb. Interestingly, the smallest 340 kb segment is shared with the region of allelic loss previously identified in ovarian carcinoma.
This finding suggests the existence of a common region of 22q, involved in the pathogenesis of these female cancers. The second prevailing type of finding was a complex pattern of low-copy-number amplifications/gains within the proximal half of 22q which were always accompanied by a loss of genetic material in the telomeric part of the chromosome. Our analysis also revealed small deletions in the centromeric region that have been previously reported as normal polymorphisms. Another aim of our project was to identify genetic heterogeneity within the studied tumors. The most remarkable finding was the presence of distinct aberrations in the two samples derived from different locations of the same large tumor.
This clearly demonstrates the co-existence of separate cell populations within the tumor mass and may reflect evolving steps of tumor progression.

303 POSTER

Bilateral breast cancer – clinical features and BRCA1, BRCA2, CHEK2 mutations

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Purpose: The aim of the study was to analyze clinical features of patients with bilateral breast cancer and to determine the contribution of BRCA1, BRCA2 and CHEK2 mutations in analyzed group.
Material and methods: Five hundred and nineteen case history of bilateral breast cancer patients (pts) treated at Cancer Center, Warsaw, Poland were analyzed and genetic mutations were evaluated in 120 of them. The time of observation was 1–56 years, median 24 years. There were 193 (34%) of synchronous (SBC) and 326 (66%) of metachronous (MBC) breast cancers. Mean age of diagnosis of SBC and MBC breast cancer was respectively 57 and 48 years ($p < 0.001$). In pts with MBC median time between detection of cancers were 5 years (range 1–54 years). Family history of breast or/ and ovarian cancer was verified in 37.5% of pts. Kaplan-Meier survival analysis was performed. Kohen-kappa homogeneity test was used regarding histological type and grade of cancers in both breasts of each patient.
Results: The probability of 5-year, 10-year and 20-year overall survival in MBC was 93%, 85% and 64% and in SBC– 82%, 71% and 46%

respectively ($p < 0.001$). Kohen-kappa homogeneity test revealed that breast cancers in each patient differed as regards histological type (MBC $p = 0.85$, SBC $p = 0.83$) but not as regards histological grade (MBC $p < 0.01$, SBC $p' < 0.001$). BRCA1 mutation was detected in 12 out of 120 patients (10%). In 8 of them Ins C was revealed. Patients with BRCA1 mutation were younger (43 vs 48 years), more often had MBC (8/12 pts) and had family history of breast cancer (55%). Eleven out of 12 patients with BRCA1 mutation live without any symptoms of the disease. BRCA2 mutation was detected in 1 (0.8%) out of 120 pts and CHEK2 mutation – in 7 (6%) out of 120 pts.
Conclusions: Cancers in two breasts of the same patient differ as regard histological type. Overall survival of bilateral breast cancer is good, but there is a significant difference between SBC and MBS. Patients with BRCA1 and BRCA2 mutation have a good prognosis. Other mutations should be searched, especially in older patients and with SBC.

304 POSTER

Prognostic profiling of node negative untreated breast cancer patients based on outcome: genomic fine-tuning

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Background: Various prognostic molecular signatures for breast cancer patients have been recently published, some of which have been recently validated on independent series, with a good prognostic value on distant recurrences.
However, all existing signatures fail to provide useful information on the type of recurrence expected, which could have significant impact on clinical management. On behalf of the TRANSBIG Network which will be initiating a large prospective trial of the clinical usefulness of genomic profiling, we initiated a study aimed at defining molecular profiles for several subgroups of patients based on their outcome.
Materials and methods: Untreated consecutive node negative breast cancer patients with available tumour samples were selected based on their outcome in two different French cancer centres (Institut Gustave Roussy and Centre René Huguenin): patients who did not relapse (NR)after minimum 10 years of follow-up, patients with a local relapse (LR), 30 patients with distant metastais before 5 years after initial diagnosis (M1), and 23 patients with distant metastasis after 5years of initial diagnosis (M2). Gene expression profiling using the Agilent technology was performed at Institut Gustave Roussy. A specific prognostic gene signature was defined for each sub-group of patients.
Results: A total of 15O patients were included in the present analysis: 63 NR (IGR = 39, CRH = 24), 33 LR (IGR = 14, CRH = 19), 30 M1 (IGR = 17, CRH = 13) and 23 M2 (IGR = 11, CRH = 12).
Discussion: Results of the comparison of molecular profiles of each sub-group and their prognostic value will be presented. This approach should provide some insight into pathways of local and metastatic recurrence and allow more accurate prediction of outcome for node-negative breast cancer patients.

Publication
Breast cancer – basic science, molecular predictive assays, translational research

305 PUBLICATION

Expression pattern of E cadherin in invasive ductal breast carcinoma

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Introduction: E-cadherin (E-CD) is considered to be the most important cell adhesion molecule in mammary gland. Some studies suggest that downregulation of E-CD and subsequent loss of cellular adhesiveness correlate with poor prognosis and metastasis but this is not confirmed by other studies. Many investigations suggest that E-CD protein is not expressed in invasive lobular in comparison with invasive ductal carcinoma (IDC) of the breast. A few papers report that E-CD mediated cell adhesion system can be disrupted by oncoprotein c-erbB-2/HER-2/neu in c-erbB-2-positive breast carcinomas despite ductal or lobular type.
Purpose of study: To evaluate the expression patternt of E-CD and relationship with the status of HER-2/neu in IDC and analyze an association with lymph node positivity.
Methods: We reviewed 91 cases of IDC. All cases were examined in our laboratory for suspicion for c-erbB-2 overexpression. Nottingham histologic grade, immunohistochemical staining for estrogen and progesterone receptors (ER and PR), proliferating cell nuclear antigen (PCNA), E-CD; fluorescense in situ hybridization for HER-2/neu gene amplification; and lymph node positivity were evaluated.
Results: HER-2/neu gene amplification was observed in 60.5% of IDC and positively correlated with higher histological grade and lymph node positivity. Strong positivity for PCNA was observed in 84.2% of IDC and positively correlated with histological grade and HER-2/neu positivity. IDCs were negative for ER in 50%, and PR in 57.8% of cases. ER/PR-negativity was associated with histological grade, HER-2/neu gene amplification and lymph node positivity. E-CD expression was lost in 26.3% cases of IDCs and positively correlated with histological grade, HER-2/neu gene amplification and lymph node positivity.
Conclusion: The loss of E-CD expression can be a feature of some typical invasive ductal carcinomas of the breast. E-CD negativity seems to be associated with higher histological grade, HER-2/neu gene amplification and lymph node positivity suggesting that c-erbB-2 may act as a regulator of E-CD expression in most human breast carcinomas *in vivo*.
This work was supported by grants NR7844–3 and MSM6198959216.

306 PUBLICATION

The immunohistochemical expression of estrogen receptor beta in breast cancer and its correlation with selected clinicopathological parameters and with survival

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Background: The role of estrogen (ER) and progesterone receptors (PgR) in breast cancer is well established. Recently, another type of estrogen receptor, termed ERβ has been discovered. The "classical" ER is now called estrogen receptor α (ERα). While ERα and PgR assays have been routinely used for a number of years, the role of ERβ is still undefined. The aim of this work was to determine the extent of ERα, ERβ and PgR immunohistochemical expression in breast cancer and to determine if the ERβ expression is correlated with selected clinical parameters, biological markers and with survival.
Methods: Formalin-fixed, paraffin embedded breast cancer tissues used in our study came from 110 women who had undergone surgery at our department between 1998–1999. None of the patients had been treated pre-operatively with endocrine therapy. Immunostaining for ERα, ERβ and PgR was performed using monoclonal antibodies against ERα, PgR (DakoCytomation), and against ERβ (CHEMICON). The EnVision detection system was applied. The data were analyzed using a nonparametric Fisher-Freeman-Halton test and log-rang test for disease-free survival (DFS) and overall survival (OS). The statistical significance was considered when $p < 0.05$.
Results: 61% of tumors were ERα positive, 64% were PgR positive and 55% were ERβ positive. As many as 14% of ERβ positive tumors had no expression of ERα. In tumors expressing ERβ, the expression of p53 was less common and ERβ positive tumors were of a lower histological grade. There was no correlation between ERβ expression and tumor size and axillary node involvement. Patients with tumors expressing ERβ had better DFS (5 years follow-up), but there was no statistically significant difference in OS.
Conclusions: The expression of ERβ was significant in breast cancer and was also present in a noticeable proportion of ERα negative tumors. Future studies will be required to determine the clinical significance of ERβ in breast cancer.

307 PUBLICATION

The expression of CCR7 in breast cancer tissue and CCL21 in lymph node does not correlate with sentinel node metastasis

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Background: Lymph node metastasis is a major prognostic factor for breast cancer patients. Sentinel node (SN) is defined as the nearest lymph node(s) from primary tumor, however, the factor(s) that can affect on SN metastasis has not been elucidated yet. On the other hand, some types of chemokines have been known to correlate with breast cancer metastasis. Among them, CC chemokine receptor7 (CCR7) is expressed on breast cancer cells, and the CCR7 ligand CCL21 is expressed selectively in lymph nodes. The aim of the present study was to examine the relationship between CCR7 protein expression of primary breast cancer and CCL21 expression of lymph nodes, including SN, and to explore whether CCR7 and/or CCL21 expression in breast cancer patient correlate with SN metastasis.