Developments in next-generation sequencing (NGS) technology have allowed the high resolution interrogation of the genomes of large numbers of colorectal cancers (CRCs). Most potential driver genes have been identified in CRCs and the challenge now is to derive clinical utility from molecular data. In addition, the functional interactions and downstream targets of the driver genes must be investigated to identify new therapeutic avenues. We have used targeted NGS (for a panel of 26 known cancer driver genes) to investigate the utility of diagnostic biopsies for predictive testing. Our data suggest that even though the biopsies represent a tiny portion of the tumour, they are sufficiently representative for evaluation of genes mutated early in the adenoma-carcinoma sequence. We have recently shown that a significant proportion of tumours thought to have chromosomal instability are, actually, chromosomally stable and form a third genetic pathway called microsatellite and chromosomal stable (MACS). CRCs with chromosomal instability (CIN) were compared with MACS CRCs using the same gene panel; however, a specific difference did not emerge. Comparison of primary tumours with their corresponding metastases similarly did not identify a specific metastasis-associated mutation. We have investigated the functional interaction of Wnt signalling, Kras and STAT3 signalling. We found that, counterintuitively, Wnt signalling was a negative regulator of Kras signalling but both pathways worked synergistically to promote cell proliferation and cell motility. In contrast, STAT3 was a positive regulator of Wnt signalling (and, through this, a negative regulator of Kras signalling). However, despite up-regulating Wnt signalling, the STAT3 functioned to inhibit the proliferative activity of Wnt signalling. We conclude that, in the post NGS world, data need to be managed to optimize patient management and that functional testing of the driver mutations identified by NGS shows interesting and unexpected interactions.

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THE EFFECTS OF RAPAMYCINE ON LUNG CANCER CELL LINE THROUGH FOXO AND PTEN
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Lung cancer or pulmonary carcinoma is the most common cause of cancer-related death in men and women. As an inhibitor of mTORC1, rapamycin, is a macrolide produced by the bacteria Streptomyces hygroscopicus and has potent immunosuppressive and antiproliferative properties. FOX (Forkhead box) proteins are a family of transcription factors that play important roles in regulating the expression of genes involved in cell growth, proliferation and differentiation. Phosphatase and tensin homolog (PTEN) is an important protein and mutations of the gene encoding it are important in the development of many cancers. In this study, we have investigated the effects of rapamycin on the distribution of FOXO-1, FOXO-3a and PTEN in the A427 human lung cancer cell line by using an indirect immunohistochemical method. A427 human lung cancer cells were grown in MEM medium with 10% fetal bovine serum, 2% L-Glutamine, 2% antibiotic, 5% CO₂ at 37°C in a humid incubator. The study was carried out under 2 groups; control group (A427 human lung cancer cells) and rapamycin group. The effects of rapamycin at 24 hours were evaluated. After fixing the cells with paraformaldehyde, the avidin-biotin-peroxidase method was employed by using anti-FOXO-1, anti-FOXO-3a and anti-PTEN primary antibodies. The immunohistochemical distribution intensities of primary antibodies were scored as mild, moderate or strong and analysed comparatively by using the ANOVA statistical test. It was observed that rapamycin had antiproliferative effects on the A427 human lung cancer cell line as assessed by immunohistochemical evaluation under a light microscope. Immunoreactivities of FOXO-1, FOXO-3a and PTEN were observed as moderate/strong/strong in the control group (A427); mild/moderate/mild in the rapamycin group, respectively. In the rapamycin group, the immunoreactivity of FOXO-1, FOXO-3a and PTEN statistically decreased compared to the control group (p<0.05). Rapamycin, in addition to anti-chemotherapeutic therapy, might be effective on the antiproliferative pathways. Inhibition of mTORC1 might be an effective target for prevention of tumor invasion and metastasis in lung cancer patients.

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THE GENETIC LANDSCAPE OF FAMILIAL BREAST CANCER IN ICELAND

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In Iceland a rare founder mutation has been detected in BRCA1 and a frequent one in BRCA2. An extensive analysis on somatic changes and gene expression in tumours of the BRCA2 999del5 founder-mutation carriers has been made. Population-based studies have been performed on a large number of available carriers, regarding penetrance, cancer risk, phenotype, clinicopathology, genetic modifiers and influence from the environment. Introduction: Genetic background is of importance for breast-cancer development and gene variants are many with both high and low penetrance. Genomic instability has a role in breast cancer pathogenesis, particularly in hereditary breast cancer, and affects sensitivity and resistance to therapy. Somatic events in tumours of individuals
with germline mutation in breast cancer-predisposing genes are fundamental for breast tumour pathogenesis. In carriers of BRCA1 and BRCA2 mutations there is an elevated risk increase of breast cancer and some other cancer types. Most of the mutations involved are of relatively high penetrance. Founder mutations in breast cancer genes such as BRCA1 or BRCA2 permit analysis of a large number of cases that have the same mutation. When founder mutations have been identified it is possible to examine the prevalence of mutations in different populations and mutation-specific effects on penetrance and disease phenotype. This information can be useful in understanding the role played by these genes in the incidence of breast cancer in order to target genetic testing, provide individual risk assessment and design better therapeutic strategies. This review will focus on BRCA1 and BRCA2 mutation carriers, mainly the Icelandic BRCA2 999del5 founder mutation. Icelandic founder mutations in BRCA1 and BRCA2. The Icelandic population originated about 1,100 years ago, comprising Nordic and Celtic settlers. The number of primary settlers was small and the population fluctuated over the centuries between 40,000 and 60,000 until the mid-19th century. Several times the population has been adversely affected by cold winters, epidemics and ephephs from volcanic eruptions. Due to improved living standards the population has since risen rapidly, especially in the past 100 years, and the population today is 326,000. This background is conducive to an enhanced probability of founder mutations. To date only one mutation in each of the BRCA1 and BRCA2 genes has been identified in the Icelandic population. In the case of the BRCA1 gene this is a rare mutation and in the BRCA2 gene a common one; both are deemed to be of founder origin (1, 2). Due to relatively high frequency, the Icelandic BRCA2 999del5 founder mutation has received the most attention, and has been analysed by several approaches. The most important findings are listed in Figure. Population-based studies, cancer risk and patient survival in relation to BRCA2 999del5 carriers. Iceland keeps detailed health and genealogy records on its genetically isolated population and, in more recent times, also on lifestyle and risk factors (8). Sample collection and genome analysis on Icelanders has proven to be an important research tool as it is drawn from population-based series of cancer cases. The original estimation of cancer risk of BRCA1 and BRCA2 mutation carriers was based on families with a high predisposition to breast cancer. Hence, mutation penetrance was relatively high. Also, in these studies different mutations in one of the genes were pooled. Today more precise data are available on penetrance, including the BRCA2 999del5 Icelandic founder mutation. This mutation is present in 8% of unselected breast cancer patients in Iceland and in 24% of women diagnosed before the age of 40 years (6). The estimated breast cancer risk in BRCA2 999del5 carriers at the age of 70 years is about 40% (7). More recent data show that the breast cancer risk has increased in recent generations (8). The cumulative incidence of breast cancer before the age of 70 years in BRCA2 999del5 carriers was detected as 19% in 1920 and 72% in 2002 (8). These observations imply that non-genetic factors may modify the inherited risk through changes in lifestyle. Breast cancer patients who carry the Icelandic BRCA2 999del5 mutation have poorer long-term survival than non-carriers but the adverse prognosis is restricted to mutation carriers with diploid, slowly-proliferating tumours (13). These data suggest that ER-status, S-phase and diploidy define two distinct subclasses of breast cancers among BRCA2 999del5 mutation carriers and that women with ER-positive, diploid cancers of low proliferation are at a high risk of recurrence and death. In addition, the Icelandic BRCA2 founder mutation is associated with a highly aggressive and rapidly-progressing form of lethal prostate cancer (12). Pathology, gene expression and somatic events in BRCA2 999del5 tumours. In general, BRCA1 and BRCA2 tumours have more aggressive phenotype than sporadic tumours, as indicated by S-phase, mitosis, aneuploidy, genomic instability and pathological appearance. Accordingly, the breast cancers associated with the BRCA2 999del5 mutation are high-grade tumors with a rapid proliferation rate (18). Also characteristic of BRCA1 tumours is the appearance of medullary phenotype since most of them have been classed as triple-negative because of the lack of expression of ER, PR or HER2. These tumours also show a high expression of basal-cell markers and loss of expression of luminal cytokeratins. The biomarker pattern of BRCA2-mutated cancers is more heterogeneous, showing a trend to positivity for hormone receptors. Most BRCA2 999del5 tumours express luminal markers, of which the majority subcategorize as luminal B and show low expression of HER2 (22). The gross genomic instability detected in BRCA1 and BRCA2 tumours fits well with their documented function in DNA repair (15, 16, 21). The chromosome aberration profiles of BRCA1 and BRCA2 tumours differ from each other and from other breast cancers, suggesting that specific genetic pathways operate in the progression of genomic instability in these inherited tumours. Functional support for discrimination between BRCA1, BRCA2 and sporadic breast tumours is also evident from genome-wide gene expression profiles. Furthermore, by subgrouping the BRCA2 999del5 tumours to luminal A, luminal B and basal/triple-negative phenotype, the pattern of chromosome aberration is different in luminal vs. basal/triple-negative phenotype tumours (22). In BRCA2 999del5 carriers, tetraploidy is associated with luminal characteristics (23). Somatic events and changes in expression of oncogenes and tumour-suppressor genes have been analysed in breast tumours of BRCA2 999del5 carriers. AURKA amplification is found in 22% of noncarriers and is more frequent in BRCA2 999del5 carriers, or 70% (20). The TP53 and CHK2 pathways are altered in BRCA1 and BRCA2 mutation carriers and
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BRCA1 carcinomas frequently carry TP53 and CHK2 mutations. This is probably due to selection during the malignant progression in the context of insufficient DNA repair, in the form of control of cell turnover, preliminary crisis phase and apoptosis. While TP53 mutations are not as frequent in BRCA2- as in BRCA1-associated tumours, overexpression of p53 is detected, suggesting that in BRCA2 999del5 mutation-carriers the p53 pathway is deregulated (17). Similarly, up-regulation of p16 and down-regulation of pRb is detected in the triple-negative phenotype of BRCA2 999del5 carriers and cyclinD1 is up-regulated in the luminal subtypes (22). Also, the FHIT gene, located at the most common fragile site in the human genome, is frequently altered and down-regulated in BRCA2 999del5 breast cancer (19). Low-penetrance genes and BRCA2 999del5 mutation carriers. Several rare and common variants, some but not all within known genes, have been described in the Icelandic population in relation to breast cancer risk. Most of them are low- or moderate-penetrance variants and are beyond the scope of this review. Nonetheless, some attention will be paid to genetic variants of breast cancer susceptibility genes that have been analysed in BRCA2 999del5 carriers, particularly those that seem to modify the BRCA2 mutation phenotype. A population-based cohort of Icelandic breast-cancer patients suggests that there is a minor elevation in risk of breast cancer in BARD1 C557S carriers, which is further elevated in BRCA2 999del5 carriers (10). This is the opposite of the low penetrance alleles of CHK2 and AURKA, T59K and F31I respectively (24, 25). In both these cases there is an increased risk of breast cancer in carriers but not in the BRCA2 999del5 context (24, 25). When looking for low-penetrance cancer-susceptibility genes it is important to acknowledge the influence of BRCA1 and BRCA2. Likewise, it is important to know the status of low-penetrance genes when estimating the penetrance of BRCA1 and BRCA2. A possible explanation could be that certain gene variants entail less growth advantage of breast tumours in BRCA2 carriers, while others confer increased growth advantage, influencing cancer progression. Conclusion: Information on new breast-cancer genes is expected in the near future and genetic association studies, which survey the entire genome, are being developed in order to uncover the genetic basis of breast cancer. Such studies have identified several novel loci, including common variants on different chromosomes, some of which may modify the BRCA1 and BRCA2 mutation phenotype. Massive databases are now available of Icelandic genomes, tracking
millions of variants across thousands of individuals. Further work should be done to link these variants to clinical histories. It will be of interest to analyse breast tumours on the level of whole genomes. Molecular pathways need to be clarified further. New information is currently under consideration for developing therapy strategies in hereditary breast cancer. This information also includes the somatic events in hereditary breast cancer. It will be important to compare the findings on the Icelandic BRCA2 founder mutation with other populations and to study the impacts of various treatments on carriers.


ASSOCIATION BETWEEN ERCC2 GENE POLYMORPHISM AND PRIMARY OVARIAN CANCER

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Introduction: Ovarian cancer (OC) constitutes 25% of genital cancers in women and is referred as the second most common gynecological cancer in developed countries. ERCC2 (excision repair cross-complementation group 2) or XPD is a protein involved in transcription-coupled nucleotide excision repair. The ERCC2 gene encodes for a 2.3-kb mRNA containing 22 exons and 21 introns. The XPD protein is a 760 amino acid polypeptide with a size of 87 kDa. The aim of this study was to evaluate the role of polymorphism of ERCC2 in ovarian cancer susceptibility in a Turkish population. Materials and Methods: Genotypes of 72 patients with ovarian cancer and 70 healthy control subjects were determined using real-time PCR for evaluation of the ERCC2 gene rs13181 polymorphism. Results: The OC group consisted of 72 female participants (mean age, 46.65±12.857) and the control group consisted of 70 female participants (mean age, 40.48±14.014). The genotype frequencies of the ERCC2 gene polymorphism among study group are 9.5% TT, 43.2% GG, 47.3% TG in the patient group and 18.4% TT, 40.8% GG, 40.8% TC in the control group, respectively. The frequency of the wild type T allele absence was found 59.5%, presence 40.5% in patient group and T allele absence was found 59.2% and presence 40.8% in control group. The frequency of the mutant G allele absence was found to be 90.5%, presence 9.5% in the patient group and G allele presence was found 81.6%, presence 18.4% in the control group. There were no significant differences between ERCC2 genotypes and alleles in the study groups. Discussion: In this study, we examined the ERCC2 gene rs13181 polymorphism and our findings showed that there was no association with ovarian cancer outcome. We consider that determination of ERCC2 gene polymorphism in ovarian cancer may have predictive importance in terms of both diagnosis and prognosis.