

MOLECULAR PATHOLOGY AND MOLECULAR EPIDEMIOLOGY OF BREAST AND COLORECTAL CANCER

Genome-wide genomic profiling in hereditary breast cancer (PI Petra Nederlof) Breast tumors from BRCA1 and BRCA2 mutation carriers show specific chromosomal changes, and these genomic profiles can be used to classify individual tumors. We use a genome-wide array-CGH analysis containing ~3500 1 Mb spaced BAC clones with routinely formalin-fixed paraffin embedded (FFPE) tumor material. We showed that basal like sporadic tumors frequently show a BRCA1-like aCGH pattern, in combination with methylation of the BRCA1 promoter and a TP53 mutation spectrum similar to that found in BRCA1 tumors. In collaboration with Dr. P Devilee (Leiden) we have analyzed tumors from BRCA1/2 negative HBC families (BRCAx). The tumors show heterogeneous aCGH profiles. Interestingly, a subgroup of tumors show a similar pattern characterized by among others gain of chromosome #22 and loss of chromosome #13. Several families showed this pattern, which now allows linkage analysis in this subpopulation. Furthermore, sporadic basal-like breast tumors resemble BRCA1 tumors in their aCGH profile, IHC profile and p53 mutations, and frequently show inactivation of BRCA1 by methylation.

Genetic determinants of breast cancer risk and breast cancer outcome

A large part of breast cancer susceptibility is as yet unexplained. Large Genome Wide Association Studies have identified new loci, which have been validated in large consortia. We validated within the Breast Cancer Association Consortium (BCAC) 18 breast cancer susceptibility loci (common single nucleotide polymorphisms) explaining ~8% of familial breast cancer. We showed that specific breast cancer risk loci (e.g. in *FGFR2*) predispose to tumors of a certain breast tumor subtype defined by immunohistochemical markers (ER, PR, HER2, CK5/6, EGFR). Furthermore, these breast tumor subtypes predispose to different breast cancer survival patterns. In our own Dutch breast cancer studies we found evidence that two functional polymorphisms *TP53* R72P (215G>C) and *MDM2* SNP309 (-4T>G) play a role in breast cancer survival within the group of patients with specific tumors. Using the Tissue Micro Arrays constructed of tumors collected in our breast cancer series diagnosed <50 years ('BOSOM' cohort), we showed that Annexin A1 is associated with a basal tumor subtype and possibly with a worse breast cancer outcome.

Validation of the 70-gene prognosis-signature in T1 breast cancer

Mammographic screening and increased awareness has led to an increase in the detection of T1 breast tumors that are generally estimated as having low risk of recurrence after locoregional treatment. However, even small tumors can metastasize, which leaves us with the question for the necessity of adjuvant treatment. Therefore, we evaluated the prognostic accuracy of the 70-gene MammaPrint signature in T1 breast cancer. In a pooled analysis of 964 breast cancer patients the 70-gene MammaPrint signature was an independent prognostic factor which can help to individualize adjuvant treatment recommendation in this increasing breast cancer population with small tumors.

Specific DNA aberrations associated with colorectal cancer metastasis

Accurate staging of colorectal cancer (CRC) with clinicopathological parameters is important for predicting prognosis and guiding treatment but provides no information about organ site of metastases. Patterns of genomic aberrations in primary colorectal tumors may reveal a chromosomal signature for organ specific metastases. Array CGH was employed to assess DNA copy number changes in primary colorectal tumors of three distinctive patient groups. A novel statistical method for identifying recurrent copy number changes, KC-SMART, was used to find specific locations of genomic aberrations specific for various groups. We created a classifier for organ specific metastases based on the aCGH data using Prediction Analysis for Microarrays (PAM). Liver specific CRC metastases may be predicted with a high accuracy based on specific genomic aberrations in the primary CRC tumor. The ability to predict the site of metastases is important for improvement of personalized patient management.



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Key publications

Blows FM, Driver KE, Schmidt MK, Broeks A, et al Huntsman D. *Subtyping of breast cancer by immunohistochemistry to investigate a relationship between subtype and short and long term survival: a collaborative analysis of data for 10,159 cases from 12 studies.* *PLoS Med* 2010;7:e1000279

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Mook S, Knauer M, Bueno-de-Mesquita JM, Retel VP, Wesseling J, Linn SC, Van 't Veer LJ, Rutgers EJ. *Metastatic potential of T1 breast cancer can be predicted by the 70-gene MammaPrint signature. Ann Surg Oncol.* 2010;17:1406-1413

Avoidance of overtreatment with radiotherapy in rectal cancer patients

The development of local recurrences (LR) is a major problem in the treatment of rectal cancer and there is a high clinical need to identify those patients who are at increased risk. In this study (collaboration C. Marijnen, LUMC), 1. we aim to develop a gene expression profile for the risk of LR in rectal cancer patients, allowing for selection of patients for pre-radiotherapy (PRT). Non-irradiated fresh frozen tumor samples from 240 stage I-III rectal cancer patients were collected from the Dutch total mesorectal excision (TME) trial, in which patients were randomized for short-term pre-RT followed by TME or TME alone. 215 samples with > 50% tumor content and high RNA quality were subjected to Illumina bead-array and gene expression analysis is in progress. 2. By using parallel extracted DNA, we have shown that *PIK3CA* mutations can be used as a biomarker in identifying rectal cancer patients with an increased risk for LR. Comparison with irradiated patients within the TME trial revealed a beneficial effect of pre-RT on *PIK3CA* mutant patients in preventing LR. Our findings suggest that *PIK3CA* mutations may not only be a prognostic factor, but may also be predictive with regard to PRT benefit. If this is confirmed in further studies, it could potentially allow the selection of patients with increased benefit from PRT versus surgery only and spare others the side effects of radiotherapy.

Quantitative Expression Profiling of RNA from FFPE and from Fresh Frozen Tissues using DASL Illumina platform

Tissues samples collected during surgery and biopsies are often fixed in formalin, followed by embedding in paraffin. Many of these samples represent clinical material with the potential to provide critical insight into expression profiles associated with complex disease as the breast cancer. This project aims to evaluate if the DASL-based expression profiling assay, specifically designed for degraded RNAs (Illumina Inc 2004) is a sensitive and reliable method to apply on RNA from FFPE. We analyzed 70 samples, 50 breast cancer samples (44 FFPE tissues and 6 FF tissues) and 20 liposarcoma samples (12 FFPE tissues and 8 FF tissues) on the 502 cancer-related genes DASL platform. The results showed that DASL platform works well with paraffin tissues, as we could see in the correlation analysis with frozen tissues (Pearson correlation Breast tissues 0.74 ± 0.05 , Liposarcoma 0.77 ± 0.04). Subsequently we enlarged the cohort of breast samples pairs and analyze them with the whole genome DASL platform. We profiled, in duplicate, 20 FFPE breast tissues and 20 matched FF breast tissues and evaluated the concordance of the DASL results from FFPE and matched FF material. We show that after proper normalization, all FFPE and FF pairs exhibit a high level of similarity (Pearson correlation > 0.7), significantly larger than the similarity between non-paired samples. Predictions of gene expression signatures developed on frozen material (Intrinsic subtype, Genomic Grade Index, 70 gene signature) showed a high level of concordance between FFPE and FF matched pairs.

Target identification and evaluation for breast molecular imaging

By visualizing molecular changes accompanying breast carcinogenesis, molecular imaging holds the promise to outperform conventional imaging modalities, possibly improving screening, diagnosis, and therapy direction. In preclinical research, many imaging probes targeting cancer-associated molecules have been successfully developed and tested. One of the hurdles for human breast molecular imaging however, is the reality that no single cancer-specific molecule is over-expressed in all breast cancers. The Breast Imaging Target Evaluation (BrITE) project aims to identify an optimal panel of imaging targets for breast molecular imaging, which would greatly expedite the development and translation of molecular imaging probes.