Karolinska Institutet

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ANALYSIS OF CHROMOSOMAL REARRANGEMENTS AND GENE COPY NUMBER CHANGES IN BREAST CANCER CELLS

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ABSTRACT

Genome changes in terms of numerical chromosomal aberrations and structural rearrangements, including deletions, amplifications and translocations, gradually accumulate in the genome during tumor development. These genomic changes are likely to play an important role in the process of tumor progression. A detailed high resolution study of genome rearrangements in breast cancer and their relation to tumor progression and risk for metastasis was performed.

A combination of the high resolution arrayCGH (aCGH) technique ROMA (Representational Oligonucleotide Microarray Analysis) and the molecular cytogenetic technique FISH (Fluorescence In Situ Hybridization) made it possible to identify genes and chromosomal regions that display aberrations as gains, losses and rearrangements. These chromosomal aberrations were analyzed quantitatively and related to the progression of the cancer disease.

Three characteristic patterns of genomic copy number variations in breast cancer were identified, simplex, complex I ("sawtooth") and complex II ("firestorm"). The simplex pattern has broad segments of duplication and deletion, usually comprising entire chromosomes or chromosome arms. The "sawtooth" pattern is characterized by many narrow segments of duplication and deletion, often alternating, more or less affecting all the chromosomes. The "firestorm" pattern resembles the simplex type except that the cancers contain at least one localized region of clustered, relatively narrow peaks of amplification, with each cluster limited to a single chromosome arm. The simplex pattern is associated with low malignant tumors whereas the complex patterns are associated with high malignant tumors. M-FISH enabled us to study the spatial organization between different chromosomal regions in the cell nucleus and validate the observed gene copy number changes quantitatively.

A method, named Sector-Ploidy-Profiling (SPP), was developed and used to compare different subpopulations from different areas in a tumor. Clonal genomic heterogeneity was found to be very common in breast cancers. The clonal subpopulations were found to be either anatomically separated or intermixed. By comparing the different subpopulations, a better understanding of the order of genomic events during tumor development could be obtained.

Inspired by the simplex and complex patterns we developed an objective estimate of genomic alterations in aCGH data. By using this method a clear relationship between genomic alterations (copy number changes and structural rearrangements) and gene expression subtypes was found.

By combining ROMA with M-FISH it became possible to study specific translocations in interphase chromatin in clinical samples. The specific translocation between chromosome 1 and chromosome 16, t(1;16), could now be visualized in both invasive breast carcinoma and in ductal carcinoma in situ (DCIS), indicating that the translocation t(1;16) is an early event during breast cancer development. In DCIS however, cells with and without translocations were found to co-exist in the same area of the tumor. This suggests that intraductal proliferation leading to DCIS precedes the translocation t(1;16).

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