INTEGRATED GENOMIC ANALYSIS OF BREAST CANCERS

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ABSTRACT

Breast cancer is the most frequent and the most
deadly cancer in women in Western countries.
Different classifications of disease (anatomoclini-
cal, pathological, prognostic, genetic) are used for
guiding the management of patients. Unfortunately,
they fail to reflect the whole clinical heterogeneity
of the disease. Consequently, molecularly distinct
diseases are grouped in similar clinical classes,
likely explaining the different clinical outcome
between patients in a given class, and the fact that
selection of the most appropriate diagnostic or
therapeutic strategy for each patient is not done ac-
curately. Today, treatment is efficient in only 70.0-
75.0% of cases overall. Our repertoire of efficient
drugs is limited but is being expanded with the
discovery of new molecular targets for new drugs,
based on the identification of candidate oncogenes
and tumor suppressor genes (TSG) functionally rel-
evant in disease. Development of new drugs makes

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therapeutical decisions even more demanding of
reliable classifiers and prognostic/predictive tests.
Breast cancer is a complex, heterogeneous disease
at the molecular level. The combinatorial molecular
origin and the heterogeneity of malignant cells, and
the variability of the host background, create dis-
tinct subgroups of tumors endowed with different
phenotypic features such as response to therapy and
clinical outcome. Cellular and molecular analyses
can identify new classes biologically and clinically
relevant, as well as provide new clinically relevant
markers and targets.

The various stages of mammary tumorigenesis
are not clearly defined and the genetic and epige-
netic events critical to the development and aggres-
siveness of breast cancer are not precisely known.
Because the phenotype of tumors is dependent on
many genes, a large-scale and integrated molecular
characterization of the genetic and epigenetic alter-
ations and gene expression deregulation should al-
low the identification of new molecular classes clin-
ically relevant, as well as among the altered genes
and/or pathways, the identification of more accurate
molecular diagnostic, prognostic/predictive factors,
and for some of them, after functional validation,
the identification of new therapeutic targets.

Keywords: Breast cancers, Genome, Transcrip-
tome, Epigenome, Oncogenes, Tumor suppressor
genes

INTRODUCTION

High-Throughput Molecular Analyses in Breast Cancer and Translational Research. Unprecedented molecular characterization is possible using high-throughput molecular analyses, available at the DNA level with comparative genomic hybridization on microarrays (aCGH) [1-4], and at the RNA level, for expression profiling with DNA microarrays [5]. When these techniques emerged, expected applications were multiple in oncology, in both basic and translational research.

A number of studies have already shown the promising role of DNA microarray-based expression profiling in breast cancer translational research by identifying new clinically and biologically relevant intrinsic molecular subtypes (luminal A, luminal B, ERBB2+, basal, and normal-like) [6-7] and new prognostic and/or predictive gene signatures, whose predictive impact is superior to conventional histoclinical factors (for review, see [8]). Currently, three prognostic gene signatures are already commercially available: Oncotype DX (Genomic Health, Inc., Redwood City, CA, USA), MammaPrint (Agendia BV, Amsterdam, The Netherlands), and the HOXB13/ IL17BR (H/I) ratio (Theros H/ISM; bioTheranostics, San Diego, CA, USA). Others under development include the Intrinsic Gene Set, the Rotterdam Signature, the Wound Response Indicator, and the Invasive Gene Signature. Similarly, signatures predictive for response to specific therapies have been reported [9-12]. These prognostic or predictive signatures, once prospectively validated, will provide the opportunity to refine our therapeutic approach by individualizing treatment to patients' individual tumor profiles, likely contributing to significantly improve the clinical outcome (for review, see [13]).

The aCGH technology has been applied more recently to breast cancer. To date, some studies, including ours, have suggested a prognostic role of genomic data [14-16]. The integrative analysis of whole-genome expression and genomic data has revealed promising results for identifying candidate genes (identified as deregulated at the DNA and RNA levels simultaneously) associated with breast cancer or with specific features of disease [14,16-24].

For years, our laboratory has identified a large number of molecular alterations in recurrent breast cancer associated with: *i)* structural aberrations such as breakages [25-29], and *ii)* evaluated the clinical impact of the amplification [14,30,31]. We were among the first to demonstrate that the integrative analysis of whole-genome expression and genomic high resolution data are useful to identify new oncogenes and TSG specific to a clinical entity or a molecular subtype. Therefore, our comparative analyses of integrated profiles of breast cancers have been reported in basal and luminal tumors, two molecular subtypes of very different clinical courses [19], but also in particularly aggressive cancer: inflammatory breast cancer [32], breast cancers in young women (Raynaud *et al.,* in preparation), and *ERBB2* amplified breast cancers [33]. This laboratory was also one of the first to identify specific genomic markers of luminal B: *L3MBTL4* (18p11) [34] and *ZNF703* (8p12) [35] as potential TSG and oncogene, respectively.

Candidate Genes May Also be Transcriptionally Deregulated Because of Epigenetic Alterations. The widespread deregulation of basic epigenetic profiles has emerged as a common phenotypic trait of cancer cells [36-38]. The epigenetic modifications include covalent tags added to nucleosome histone components [*e.g.,* acetylation of histone H3 and/or H4 (H3/4Ac) and/or various levels of methylation on lysine residues of histone H3 (H3K4/ K9me1/ 2/3), a non exhaustive list defined as the histone code], as well as methylation of CpG dinucleotides [39,40]. This applies particularly to CpG methylation profiles, whose modification has direct implication on many aspects of cell biology, namely cell division, survival, development and, consequently, oncogenesis. DNA methylation at regulatory regions of a gene, including promoter, generally leads to transcriptional silencing. CpG methylation-dependent silencing is now considered as an important mechanism of TSG inactivation in cancer cells, in addition to somatic genetic lesions [41]. DNA methylation changes in human cancers are complex and vary between different tumor types. Promoter methylation effectively represses transcription and occurs in many genes involved in human breast cancer development [42]. Among these, genes associated with cell cycle regulation (APC, RASSF1, RB, TFAP2A), or coding for steroid receptors (ESR1, PGR, RARα), suppressors (BRCA1, CDKN2A, CST6), and genes associated

with metastasis (CDH1, CEACAM6, PCDHGB6) and other genes such as *NRG1*. The majority of these affected genes are potential or known TSG [43]. Interestingly, there is also increasing evidence that methylation of regulatory regions of cancer-related genes can be one of the most prevalent molecular markers for human cancer diseases [44]. The potential clinical applications of DNA-methylation biomarkers may include diagnosis of neoplasm, tumor classification, prediction of response to treatment, or prognosis. DNA methylation status has thus been extensively studied in various molecular or clinical entities in breast cancers in order to better characterize them or improve their molecular classification [45-49].

In the continuity of our strategy, the high resolution DNA promoter methylation status will be analyzed on human promoter array (Agilent Technologies, Massy, France) and integrated to the genomic and gene expression data previously collected in the same set of 300 breast tumors. High-throughput molecular analyses of breast cancer have already revealed some part of their potential. Such integrated approaches could contribute to better understand the various levels of the dynamic molecular changes in the mammary oncogenesis and identify new markers.

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