The inactive X chromosome is epigenetically unstable and transcriptionally labile in breast cancer.

Summary

Disappearance of the Barr body is considered a hallmark of cancer, although whether this corresponds to genetic loss or to epigenetic instability and transcriptional reactivation is unclear. Here we show that breast tumors and cell lines frequently display major epigenetic instability of the inactive X chromosome, with highly abnormal 3D nuclear organization and global perturbations of heterochromatin, including gain of euchromatic marks and aberrant distributions of repressive marks such as H3K27me3 and promoter DNA methylation. Genome-wide profiling of chromatin and transcription reveal modified epigenomic landscapes in cancer cells and a significant degree of aberrant gene activity from the inactive X chromosome, including several genes involved in cancer promotion. We demonstrate that many of these genes are aberrantly reactivated in primary breast tumors, and we further demonstrate that epigenetic instability of the inactive X can lead to perturbed dosage of X-linked factors. Taken together, our study provides the first integrated analysis of the inactive X chromosome in the context of breast cancer and establishes that epigenetic erosion of the inactive X can lead to the disappearance of the Barr body in breast cancer cells. This work offers new insights and opens up the possibility of exploiting the inactive X chromosome as an epigenetic biomarker at the molecular and cytological levels in cancer.

Histo-genomic stratification reveals the frequent amplification/overexpression of CCNE1 and BRD4 genes in non-BRCA1/2 high grade ovarian carcinoma.

Summary

The treatment of epithelial ovarian cancer (EOC) is narrowly focused despite the heterogeneity of this disease in which outcomes remain poor. To stratify EOC patients for targeted therapy, we developed an approach integrating expression and genomic analyses including the BRCA1/2 status. Gene expression and genomic profiling were used to identify genes recurrently (>5%) amplified and overexpressed in 105 EOC. The LST (Large-scale State Transition) genomic signature of BRCA1/2 was applied to define molecular subgroups of EOC. Amplified/overexpressed genes clustered mainly in 3q, 8q, 19p and 19q. These changes were generally found mutually exclusive. In the 85 patients for which the genomic
signature could be determined, genomic BRCAness was found in 52 cases (61.1%) and non-
BRCAness in 33 (38.8%). A striking mutual exclusivity was observed between BRCAness and
amplification/overexpression data. Whereas 3q and 8q alterations were preferentially
observed in BRCAness EOC, most alterations on chromosome 19 were in non-BRCAness
cases. CCNE1 (19q12) and BRD4 (19p13.1) amplification/overexpression was found in 19/33
(57.5%) of non-BRCAness cases. Such disequilibrium was also found in the TCGA EOC data
set used for validation. Potential target genes are frequently amplified/overexpressed in non-
BRCAness EOC. We report that BRD4, already identified as a target in several tumor models,
is a new potential target in high grade non-BRCAness ovarian carcinoma.

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Oct 14)

Circulating tumor DNA and circulating tumor cells in metastatic triple negative
breast cancer patients.

Summary

Circulating tumor DNA (ctDNA) is a new circulating tumor biomarker which might be used as
a prognostic biomarker in a way similar to circulating tumor cells (CTCs). Here, we used the
high prevalence of TP53 mutations in triple negative breast cancer (TNBC) to compare ctDNA
and CTC detection rates and prognostic value in metastatic TNBC patients. Forty patients
were enrolled before starting a new line of treatment. TP53 mutations were characterized in
archived tumor tissues and in plasma DNA using two next generation sequencing (NGS)
platforms in parallel. Archived tumor tissue was sequenced successfully for 31/40 patients.
TP53 mutations were found in 26/31 (84%) of tumor samples. The same mutation was
detected in the matched plasma of 21/26 (81%) patients with an additional mutation found
only in the plasma for one patient. Mutated allele fractions ranged from 2 to 70% (median
5%). The observed correlation between the two NGS approaches (R(2) = 0.903) suggested
that ctDNA levels data were quantitative. Among the 27 patients with TP53 mutations, CTC
count was ≥1 in 19 patients (70%) and ≥5 in 14 patients (52%). ctDNA levels had no
prognostic impact on time to progression (TTP) or overall survival (OS), whereas CTC
numbers were correlated with OS (p = 0.04) and marginally with TTP (p = 0.06). Performance
status and elevated LDH also had significant prognostic impact. Here, absence of prognostic
impact of baseline ctDNA level suggests that mechanisms of ctDNA release in metastatic
TNBC may involve, beyond tumor burden, biological features that do not dramatically affect
patient outcome.

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**Circulating tumor DNA as a non-invasive substitute to metastasis biopsy for tumor genotyping and personalized medicine in a prospective trial across all tumor types.**

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**Summary**

Cell-free tumor DNA (ctDNA) has the potential to enable non-invasive diagnostic tests for personalized medicine in providing similar molecular information as that derived from invasive tumor biopsies. The histology-independent phase II SHIVA trial matches patients with targeted therapeutics based on previous screening of multiple somatic mutations using metastatic biopsies. To evaluate the utility of ctDNA in this trial, as an ancillary study we performed de novo detection of somatic mutations using plasma DNA compared to metastasis biopsies in 34 patients covering 18 different tumor types, scanning 46 genes and more than 6800 COSMIC mutations with a multiplexed next-generation sequencing panel. In 27 patients, 28 of 29 mutations identified in metastasis biopsies (97%) were detected in matched ctDNA. Among these 27 patients, one additional mutation was found in ctDNA only. In the seven other patients, mutation detection from metastasis biopsy failed due to inadequate biopsy material, but was successful in all plasma DNA samples providing three more potential actionable mutations. These results suggest that ctDNA analysis is a potential alternative and/or replacement to analyses using costly, harmful and lengthy tissue biopsies of metastasis, irrespective of cancer type and metastatic site, for multiplexed mutation detection in selecting personalized therapies based on the patient’s tumor genetic content.