Analysis of active oncogenic signal transduction pathways in HGS ovarian cancer
HH pathway activity associated with platinum resistance

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Introduction

Patients with ovarian cancer in general have a bad prognosis due to frequently advanced disease at diagnosis associated with limited therapeutic options, and recurring disease. Use of targeted drugs in ovarian cancer is not very advanced, one major reason being insufficient knowledge on tumor driving signaling pathways. Hence there is a strong need for a better diagnostic approach.

Signal transduction pathway analysis approach

A new diagnostic approach is being developed [Verhaegh et al. Cancer Res 2017;77(11):2936-45] that enables quantitative measurements of the functional activity status of (oncogenic) signal transduction pathways in cell and tissue samples, based on computational inference of pathway activity from measurements of mRNA levels of well-validated direct target genes of the transcription factor associated with the respective signaling pathway. mRNA levels can be measured using Affymetrix HG-U133Plus2.0 or PM microarrays or RT-qPCR.

Validation examples of pathway models

Materials and methods

Affymetrix HG-U133+PM microarrays were run on fresh frozen samples from 37 primary high grade serous (HGS) ovarian cancer patients (90 samples in total, from multiple metastatic locations). These data, and data from a number of publicly available Affymetrix datasets were analysed for signal transduction pathway activity, including Hedgehog (HH), PI3K and NFkB pathway activity.

Results

HGS ovarian cancer patient samples, Erasmus MC

Two common pathway patterns were found:
• NFkB+FOXO active, i.e. PI3K inactive
• PI3K+Hedgehog (HH) active

Patient 17, primary carboplatin/paclitaxel resistant HGS ovarian cancer, with rapid disease progression and death: HH active and PI3K active (FOXO inactive)

Public (GEO) datasets HGS

GSE32062: Advanced HGS ovarian cancer: HH and PI3K pathway combination

GSE13525: 38M2 serious ovarian cancer cell line treated with carboplatin (100μM): induction of apoptotic changes, associated with decreased HH/PI3K activity and increased FOXO/NFkB activity

Ovarian cancer cell lines

GSE15709: Cisplatin resistant variants of HGS ovarian cancer cell line A2780 have higher HH pathway activity

All samples are PI3K active (data not shown)

Translation to qPCR for routine diagnostics

The Affymetrix models were ported to an RT-qPCR platform with 96 real-time qPCR assays.

Translation from 34 target genes to 12 genes for qPCR plate

Oncosignal qPCR test procedure and cloud-based pathway activity analysis

Conclusion

Functional signal transduction pathway activity can be quantitatively measured using Philips pathway analysis on Affymetrix microarray and RT-qPCR data. Combined patient studies (including public GEO datasets) and cell line results suggest that HH/PI3K pathway activity in HGS ovarian cancer is associated with bad prognosis and platinum resistance.

Summary results
• HGS: two subtypes: HH/PI3K pathway active and FOXO/NFkB pathway active.
• HH activity associated with bad prognosis
• Patient with high HH/PI3K pathway activity: primary platinum resistance and rapid progression.
• Ovarian cancer cell line (A2780): HH/PI3K pathway activity associated with platinum resistance.
• Activity of NFkB/FOXO may be associated with less aggressive phenotype. In platinum-sensitive ovarian cancer cell line, platinum induces NFkB/FOXO activity, associated with apoptotic changes.

Discussion

HH activity causing platinum resistance has been described for gastric cancer [Yoon et al, Clin Cancer Res. 2014;20(15):3974-86].

Possible mechanism: HH transcription factor GLI induces expression of the Nucleotide Excision Repair protein XPD, which can repair platinum-induced DNA damage.

Signal transduction pathway analysis may provide valuable information to decide on targeted therapy and immune therapy choice.