Predictive gene signatures for bevacizumab and cetuximab as well as cytotoxic agents

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**Introduction**

The identification of biomarkers or gene signatures predicting tumor response to novel targeted and cytotoxic therapy will make it possible to individualize and improve the effectiveness of anticancer therapy. The FDA strongly recommends developing such companion diagnostic tests. We investigated the hypothesis that correlating the drug response with gene expression in tumor models (patient-derived) would allow the identification of gene signatures that can predict the drug response of individual tumors for the clinic. The signatures were validated with the Leave-One-Out-Cross-validation of the training set, an independent testing set, and a clinical feasibility study.

**Materials and methods**

Patient tumors, established subcutaneously by serial passage in nude mice, were characterized for their sensitivity towards 2 targeted and 10 standard cytotoxic anticancer agents. The latter included the alkylating agents cyclophosphamide, ifosfamide, mitomycin-C and cisplatin, the antimetabolites 5-FU and gemcitabine, the topoisomerase II inhibitors adriamycin and etoposide, as well as the tubulin binders paclitaxel and docetaxel. The mean number of tumors treated with any of the various drugs was 54 (range 31 – 78). The gene expression profiles of the tumor xenografts were determined using the Affymetrix HG-U133 plus 2.0 mRNA expression array representing ~38,500 human genes. Predictive gene signatures for all the 12 agents tested were identified and subsequently verified using the leave-one-out cross-validation (LOOCV) technique. In the case of 3 of the drugs, signatures were further validated using an independent set of previously untested tumors.

Tumors were considered responsive if the drugs produced an inhibition in tumor volume to a value less than 11 – 41% of vehicle control tumors (T/C %). The median cut-off for all drugs was a T/C of 25%. Using these criteria, on average 1/3 of the test tumors were classified as sensitive (responders) and 2/3 were resistant (non-responders).

**Results**

The bioinformatic analysis yielded predictive gene signatures consisting of 20 – 129 genes (mean for the 12 drugs: 87 genes). On average, the response rate of predicted responders (83%) was 2.45 fold higher than that of all test tumors (random testing, 34%). This increase in response rate, following signature-guided testing, was consistent for all agents. Conversely, 94% of the predicted non-responders (range 84 – 100%) proved to be non-responders in the nude mouse. The function of the majority of genes (59%) making up the predictive gene signatures was unknown. Genes with known function are implicated in cell proliferation, apoptosis, DNA repair, cell cycle, metabolism and transcription. In summary, the predictive gene signatures presented here for 12 cytotoxic agents can be used to substantially increase tumor response rates compared to empirical drug treatment.

Similarly, the VEGF antibody Bevaciuzumab (BV) was also evaluated in 72 tumors (colon, NSC-lung, breast and renal cancers). BV was administered i.v. once weekly for 3 weeks. Antitumor activity was evaluated as the minimum T/C value or after 28 days. We identified a gene signature consisting of 35 genes which was validated using the LOOCV and in an independent testing set. In the LOOCV, 15/47 tumors were predicted to respond, 10 of which did respond in real testing. This corresponds to an increase of the response rate from 30% (14/47) to 67%. Of the 32 predicted non-responders, 28 (88%) were resistant in real testing’s. In the independent testing set, the prediction was correct for 71% of the predicted responders and for 89% of the predicted non-responders.
the 35 genes making up the signature predicting BV response, 21 are associated with angiogenesis.

We have also developed a gene signature for Cetuximab (CTX) consisting of 21 genes. Using a T/C of < 36% as cut-off for activity, 17% (15/90) of all tumors were classed as sensitive: 7/32 NSCLC, 4/20 colon, 3/5 head and neck, 1/3 pancreas, and 1/6 gastric cancers. The search for the gene signature was restricted to tumor types where Cetuximab has an established role in the clinic (colon, head & neck, NSCLC; total of 54 tumors). In the LOOCV, 18/54 tumors were predicted to respond, 11 of which were responsive in the real testing’s. The response rate increased from 24% to 61%. Of the predicted 36 non-responders, 34 (94%) were resistant in real testings. With 1 exception, tumors with mutated k-ras did not respond to CTX.

**Conclusion**

For both marketed drugs and drugs in development, gene signatures have great potential to improve the response rates and survival time compared to empirical drug treatment. However, the gene signatures need to be further validated in clinical studies.

**References**