lack of biomarkers to assess the risk of clinically significant recurrence and progression. Better prognostic and predictive tools are required to guide clinical management and reduce overtreatment.
Materials and Methods: Patient specimens from the Mayo Clinic Radical Prostatectomy Registry were selected from a nested case–control cohort with 14 years median follow-up. RNA expression levels from FFPE tumor specimens were measured with 1.4 million feature oligonucleotide microarrays. Patients were divided into a training set (n = 359) for variable selection using cross-validated lasso logistic regression and model building with a Random Forest classifier. The final genomic-clinical classifier (GCC), a multivariate model consisting of 43 expressed markers (genes and non-coding RNAs) and pathology review Gleason score was compared to clinical variables and a multivariate clinical model (CM) combining age, PSA, Gleason score, stage and surgical margin status to predict early clinical recurrence (positive bone or CT scans within 5 years after biochemical recurrence). The receiver-operator characteristic area-under-the curve (AUC) metric was used to evaluate GCC and the clinical models in an independent validation set (n = 187) of prostatectomy patients.
Results: In the training subset, the GCC had AUC of 0.93, while Gleason and the CM models had AUC of 0.73 and 0.76, respectively. Overall in the validation set, the GCC model had an AUC of 0.77, which compared to the AUCs of 0.65 for Gleason and 0.67 for CM models in predicting clinical recurrence. However, in contrast to the clinical models only the GCC maintained consistent performance in high-risk (node negative and pT3 and/or positive margin) patients (n = 107). In this group, the GCC had a validated AUC of 0.81 whereas the Gleason and CM models had AUCs of only 0.56 and 0.66, respectively.
Conclusion: We have developed a combined genomic-clinical classifier that shows improved performance over clinical models alone for the prediction of clinical recurrence, notably in high-risk prostatectomy patients that are the most obvious candidates for adjuvant therapy. We are further testing the performance of this classifier and its usefulness in guiding decision-making for the adjuvant therapy setting in additional validation studies.

OP 16

Identification of JAK2/STAT3 as a novel therapeutic target in Kras mutant colorectal cancer models

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Background: STAT3 is activated by Janus kinases (JAKs), which are recruited and activated by numerous cytokine receptors, receptor tyrosine kinases (including EGFR) and non-receptor tyrosine kinases (such as Src). A recent study has shown that high STAT3 activation is positively associated with adverse outcome in colorectal cancer, supporting its potential role as a therapeutic target. Kras mutations occur in 40−45% of colorectal cancer (CRC) patients and confer resistance to EGFR targeted therapies. The aim of this study was to evaluate JAK2/STAT3 signalling as novel Kras synthetic lethal interactions in CRC.
Materials and Methods: STAT3 and JAK2 inhibition was obtained using siRNA and small molecule approaches. Analysis of cell viability was carried out using MTT assay, apoptosis was measured using Western blotting and Flow cytometry and migration using xCELLigence system. The isogenic KrasMT/WT HCT116 cell line model and a panel of KrasWT&MT CRC cells were used.
Results: Using different siRNA sequences, we found that silencing of STAT3 and JAK2 was lethal in KrasMT HCT116 cell line compared to its KrasWT clone, and these results were confirmed using the small molecule JAK2/STAT3 inhibitor 'cucurbitacin'. Similar data were obtained in our panel of KrasMT CRC cells. Interestingly, significant higher constitutive levels of pSTAT3 were observed in KrasMT HCT116 cells compared to its WT clone. Combination of STAT3 or JAK2 silencing with MEK1/2 inhibition or chemotherapy (5-FU, oxaliplatin) resulted in synergistic decreases in cell viability and increase in apoptosis in KrasMT HCT116 cell line and this was associated with potent increase in STAT3 activity following MEK1/2i or chemotherapy. Furthermore, STAT3 silencing resulted in strong decreases in cell migration in KrasMT HCT116 cell line.
Conclusion: These results indicate that KrasMT CRC models are dependent on JAK2/STAT3 pathway for survival. We are now further evaluating the effect of JAK2/STAT3 inhibition in combination with MEKi/chemotherapy in in vivo models.

OP 24

Somatic allelic selection in the tumor as an indicator of cancer relevance: insights from statistical mining of the Cancer Genome Atlas data

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Background: We sought to leverage the unprecedentedly rich, high-dimensional data available from the Cancer Genome Atlas (TCGA) to uncover genetic variants that are selected for somatically in tumor cell.
Materials and Methods: We hypothesized that certain genetic variants may give a proliferative advantage to the cell when be promoted somatically via copy number lesions or methylation-based silencing of a wild-type counterpart. To test for these phenomena, we integrated TCGA data from four platforms − single nucleotide polymorphism (SNP) arrays, expression arrays, methylation arrays, and "next-generation" sequencing − to query for preferential allelic selection of specific variants. Statistical tests for recurrent somatic promotion of one inherited parental SNP haplotype over another (via amplification, loss, or promoter methylation) were developed and applied to the SNP and methylation array data. Significant regions were tested for concordant effects on mRNA expression. Deep sequencing data enabled detection of somatic mutations and rare variants that may be the true targets of allelic selection.
Results: Our analysis uncovered evidence at multiple loci of strong selective pressures in the tumor environment. In many cases, copy-number aberration and promoter methylation were both utilized as selective mechanisms at the same genomic locus. Some germline cancer susceptibility variants reported by previously-published GWAS displayed signals of preferential somatic selection over their allelic counterparts, shedding light on the mechanisms-of-action for tumor predisposition loci. Furthermore, deep sequencing of the affected regions yielded new mutations in known and novel cancer-related genes. Included among the novel genes are intriguing candidates for follow-up functional studies that are now underway.
Conclusion: Our study demonstrates one approach to separating the "driver" molecular variants from the background "passenger" noise, pinpointing candidate diagnostic markers in cancer. More generally, our results exemplify insights into cancer biology that may be obtained via statistical mining of complex, multi-faceted data sets such as those generated by TCGA.

OP 75

An unbiased shRNA based lentiviral screen identifies tyrosine kinases that are important for survival and radioresistance in Head and Neck Squamous Cell Carcinoma

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Background: The radiation oncologist's interest in modulation of receptor tyrosine kinase (RTK) signaling in Head and Neck Squamous Cell Carcinoma (HNSCC) was recently invigorated by the therapeutic success of EGFR targeted therapy. Nonetheless a large part of the RTK-family remains uninvestigated. A high throughput screen was performed on two HNSCC cell lines (SCC61 and SQD9) to identify other tyrosine kinases with possible role in radioresistance or cell survival.
Materials and Methods: We used a pooled shRNAmir library containing over 270 lentiviral vectors targeting the tyrosine kinase family. In this way − after viral transduction − each cell in the cell population contained a different shRNA, knocking down the expression of a specific tyrosine kinase. Because each viral vector contains a barcode, the presence of each shRNA in the cell population could be tracked by sequencing before and after irradiation.
Results: Using this shRNA screen, we identified several tyrosine kinases with potential importance in the proliferation and/or resistance to irradiation of the SCC61 and SQD9 cell lines. Of note, FLT1 was identified as being important for radioresistance in both of our HNSCC cell lines. Expression of this kinase was demonstrated using qPCR. Its role in survival and radioresistance was validated using 2 different FLT1 siRNA's in a sulphorodamine B assay or clonogenic assay respectively. FLT1 silencing was associated with downregulation of phospo-ERK. Sunitinib malate, a TKI with known anti-FLT1 activity, resulted also in downregulated ERK-pathway activity and a corresponding decrease in cell survival. Sequencing revealed no mutations in this receptor, but overactivity could be explained by strong autocrine production of the receptors ligands VEGFA and VEGFB. In a next step, expression of FLT1 was examined in human tissue by immunohistochemistry. FLT1 expression was demonstrated in all 13 laryngeal cancers examined and autocrine ligand expression was also documented. Interestingly, the expression of FLT1 seemed to be more pronounced in the tumor than in the surrounding normal squamous epithelia.

Conclusion: We document expression of FLT1 in HNSCC and identify this kinase as a potential target for the modulation of radioresistance in this cancer. In a next step we will evaluate this kinase as a marker for response prediction after radiotherapy.

OP 85

Activation of ERBB2 signaling causes resistance to the EGFR-directed therapeutic antibody cetuximab

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Background: The epidermal growth factor receptor directed antibody, cetuximab (Cmab), is an effective clinical therapy for patients (pts) with colorectal cancer (CRC) particularly with wild type KRAS and BRAF. Treatment in all pts is limited eventually by the development of acquired resistance but little is known about the underlying mechanism.
Materials and Methods: We established 3 Cmab resistant cell lines HCC827CR, GEOCR and A431CR through the Cmab exposure. In order to determine why these were resistant to Cmab we performed genome wide copy number analyses and analysis of ERBB family ligands. Furthermore, we obtained clinical specimens from CRC pts treated with Cmab based therapy. Specimens obtained prior to therapy and at the time of Cmab resistance were evaluated.
Results: Genome-wide copy number analysis detected the localized genomic amplification in HCC827CR, which was identified as ERBB2 and confirmed using FISH. Amplification of ERBB2 was also detected in GEOCR. ERBB2 inhibition, with trastuzumab or lapatinib, restored the Cmab sensitivity in these. In sensitive cell lines, Cmab effectively inhibited growth and ERK1/2 signaling both of which were inhibited in presence of ERBB2 amplification. In contrast, despite detecting ERBB2 activation in A431CR we did not identify ERBB2 amplification. Instead we detected increased levels of the ERBB3 ligand heregulin (HRG). The disruption of ERBB2/ERBB3 herterodimerization using pertuzumab restored Cmab sensitivity in A431CR in vitro and in vivo. CRC pts with ERBB2 amplification ($n = 13$) treated with Cmab survived significantly shorter than pts without ERBB2 amplification ($n = 220$) (Median OS 89 vs 149 days, $p = 0.0013$). We also identified evidence of ERBB2 amplification at the time of acquired resistance using either tumor biopsies or by analyzing for changes in serum HER2 extracellular domain. We further analyzed plasma HRG from CRC pts (Median, 1622 pg/ml; range 0−18,045 pg/ml). Pts who achieved response to Cmab ($n = 16$) had lower HRG concentration than pts without response ($n = 49$) (Mean, 1,050 vs 3,601 pg/ml, $p < 0.001$). In addition we identified a significant increase in plasma HRG levels obtained at the time of Cmab resistance compared to pre-treatment ($p = 0.018$).
Conclusion: We identify activation of ERBB2 signaling, either through ERBB2 amplification or HRG up-regulation, as a mechanism of both de novo and acquired Cmab resistance. These results suggest that ERBB2 inhibitors, combined with Cmab, may represent a rational therapeutic strategy in Cmab-resistant cancers.