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Therapy response to CDK4/6 inhibitors is partially mediated by RNR-complexes and combined targeting result in synergistic efficacy

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Introductions: CDK4/6 inhibition is a promising approach for treatment of BLCA but molecular mechanisms of acquired resistance are still unclear. We combined comparative proteomics and a genome scale CRISPR-dCas9 screen to identify novel treatment strategies.
Materials and methods: Protein changes in RT112 and T24 cells treated with Palbociclib, a CDK4/6 inhibitor, were analyzed using a quantitative label-free LC-MS/MS-based proteome analysis (ANOVA p_{FRR}-value ≤0.05, fold change of ≥1.5). In parallel, genes of resistance were identified using a genome-scale CRISPR-dCas9 screen using NGS and MAGeCK-VISPR. Molecular targets revealed by integrative analysis of both screens were validated on mRNA and protein level. Small molecule inhibitors against the proteasome system (MG-132, Epoxomicin, MLN4924), RNR complex (Gemcitabine, COH29), Rapalogues and siRNAs (E2F3, S6K1) were used. We monitored clonogenic growth, cell viability and tumor growth in a xenograft model. For identification of combination therapies, the combination index was applied. Western blotting was used for protein analysis.
Results: RRM2 (Ribonucleoside-diphosphate reductase subunit M2), was identified as a target that confers resistance to Palbociclib in both screens. Transcription and protein stability is synchronized to therapy response and regulated by proteasome activity and Palbociclib dependent regulation of S6K1 and E2F3. We identified a novel combination therapy with the RNR inhibitor COH29 (not Gemcitabine) that was also effective in Gemcitabine resistant cell lines.
Conclusions: Application of comparative proteomics and genome-scale transcriptional activation screens allow to identify molecular mechanisms of resistance to treatment and improve on standard therapy regimen.

V36.3

Tumor immune microenvironment drives prognostic relevance correlating with bladder cancer subtypes

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Background: Muscle-invasive bladder cancer (MIBC) represents approximately two thirds of invasive urothelial bladder cancers (UBC). We conducted this study to gain further insights in the immunological tumor microenvironment (TIME)
Material and methods: sTILs were scored on HE slides in 135 MIBC patients treated by radical cystectomy according to current recommendations. We assessed intrinsic subtypes (MDACC-approach), tertiary lymph structures (IHC), spatial immune cell distributions on regionally designed TMAs (CD3, CD8, CD56, CD68, PD-1 and PD-L1). Results were validated in 407 MIBC of the TCGA cohort by hierarchical clustering analysis, immune cell population analysis via CIBERSORT and sTIL-scoring.
Results: Quantity and spatial distribution of stromal tumor infiltrating lymphocytes (sTILs) predict stages of tumor inflammation, subtypes, patient survival and correlate with expression of immune checkpoints. High sTILs indicate an inflamed subtype with 80% 5-year disease-specific survival. A lack of immune infiltrates identifies an uninflamed subtype with a survival rate of less than 25%. A separate immune evading phenotype with upregulated immune checkpoints associated with poor survival. High TLS amounts and close tumor distance correlated significantly with an inflamed phenotype and favorable survival. High inflammation also correlated with increased neoantigen load, high TMB and specific mutational patterns. Patients treated with adjuvant chemotherapy showed a favorable prognosis dependent on high sTILs.
Conclusion: Determination of sTILs and tumor subtypes may stratify therapy success and patient survival. sTILs can easily be quantified on HE slides and couldbe implemented for predicting outcome after cystectomy and adjuvant platinum based chemotherapy.

V36.4

Medikamentöse Tumortherapie für das muskelinvasive Blasenkarzinom basierend auf dessen genetischen Profil

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Einleitung: Die biologische Heterogenität des muskelinvasiven Blasenkarzinoms (BK) ist sehr wahrscheinlich dafür verantwortlich, dass nicht eine Therapie bei allen BK effektiv ist. Unser Ziel war die Entwicklung eines Algorithmus, welcher in-silico anhand des genetischen Profils eine individuelle Therapie bestimmen kann.
Material/Methoden: DNA- und RNA-Seq Daten der TCGA BK-Kohorte dienten als Grundlage. Basierend auf Mutationen, wurden Gen-Medikament Interaktionen identifiziert, welche gemäss Relevanz geordnet wurden (Zulassung des Medikaments, bekannte Pathogenität, Daten aus klinischen Studien, Prävalenz der Mutation). Durch Integration von RNA-Seq in ein lineares Modell, wurden „drug-response-scores" (DRS) errechnet. Diese DRS wurden den mutationsbasierten Targets zur weiteren Stratifizierung hinzugefügt.
Ergebnisse: Basierend auf unseren Filterkriterien, wurden 44 Gene identifiziert, welche mit 63, in Malignomen bereits untersuchten, Medikamenten assoziiert wurden. Mittels DRS konnte bei 21 Medikamenten die Selektion weiter stratifiziert werden. Unter diesen Medikamenten befanden sich beim BK bereits eingesetzte Substanzen (Carboplatin oder Gemcitabine), sowie bisher nicht getestete Medikamente, wie z. B. Olaparib (PARP-Inhibitor), Crizotinib (ALK-TKI) oder Trametinib (Kinase-Inhibitor). Mit Hilfe unseres Algorithmus gelang in 86 % der BK eine vielversprechende, personalisierte Medikamentenselektion.
Schlussfolgerung: Basierend auf der genetischen Landschaft können bekannte und neue Therapeutika für das BK identifiziert werden. Durch Integration von DNA und RNA gelingt eine gewichtete Identifikation von Medikamenten für den einzelnen Patienten. Die funktionelle Testung in Modellen ist der nächste Schritt dieser personalisierten in-silico Medikamentenselektion.