A006

Preclinical models from rare aggressive tumors: Establishment, drug sensitivity and genomic analyses of mesothelioma PDX as models for oncology drug development

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Background and aim

Malignant mesothelioma is a rare but aggressive cancer with few therapeutic options, a poor prognosis and with expected increasing incidence. The main cause of the disease is the exposition to asbestos even after 50 years of latency, suggesting multiple somatic genomic events being required for tumorigenic conversion of a mesothelial cell. Intended therapeutic goals, such as increase of patient survival and improvement of quality of live have only been achieved to a limited extent. Curative treatment is currently not possible. Therefore, there is a strong clinical need for preclinical and translational models.

Methods

For PDX establishment, tumor tissue from surgically resected pleural or peritoneal mesotheliomas was collected from consenting patients, implanted subcutaneously into immunodeficient mice and serially passaged for at least 3 *in vivo*-passages. The stable growing PDX were characterized towards sensitivity to the standard of care (SOC) drugs pemetrexed, cisplatin, and gemcitabine as well as to two combinations thereof. In parallel, we initialized a comprehensive molecular characterization starting with RNASeq analysis which was performed by Illumina® 80 Mio read paired-end sequencing of a TrueSeq® stranded mRNA library. Genome-wide gene expression was calculated from STAR-generated read counts using the *DESeq2* R-package. Expressed sequence variations were called using GATK4 and were interpreted by means of the Ensemble VEP.

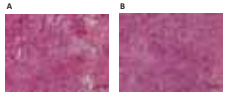
Results

We succeeded in establishing a small set of 6 mesothelioma PDX of a total of 34 clinical samples received, giving a take rate of 18%. 2 of them originate from the pleural and 4 from the peritoneal area. Histological examination revealed a high similarity between patient tumor and the respective PDX.

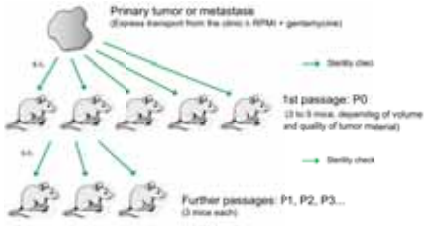
After treatment of all PDX models with 3 standard chemotherapeutic drugs and 2 combinations, we could observe very individual response rates ranging from strong to non-response but where, the combination of cisplatin with pemetrexed or gemcitabine revealed a stronger tumor growth inhibition than the respective monotherapies.

A genome-wide gene expression analysis of RNASeq read counts revealed 3 distinct clusters that might reflect the known histological subtypes. A deep analysis of expressed sequence variations found by the RNASeq variant calling resulted in 162 protein-changing mutations in 142 COSMIC-listed cancer-related genes; all of them annotated in SNPdb. Among these genes there are 6 putative drivers including one - ***SETD2*** - which is the forth most mutated gene listed in the TCGA mesothelioma study (TCGA-MESO). 3 further truncated driver genes (***JAK1***, ***LATS1***, and ***TNFRSF14***) suggest potential druggability; for ***JAK1*** targeted cancer therapeutics are under investigation. A parallel exome analysis is still ongoing.

PDX establishment



Meso7212 patient & P6: The histology of the original patient tumor (A) remains stable in the PDX derived thereof (B), even after several passages.

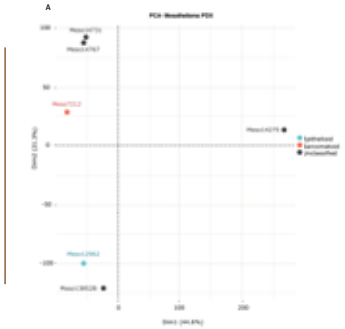


After surgery, pieces of patients tumor are express shipped and transplanted s.c. onto highly immunodeficient NOG mice immediately upon arrival. Several passages are performed until a stable growing human xenograft has developed. Tumors are maintained either *in vivo* by passaging or cryogenically banked in liquid nitrogen.

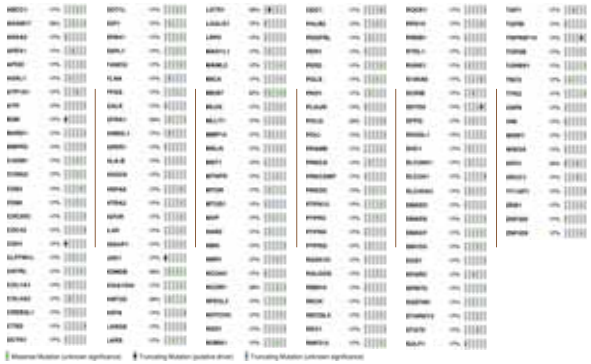
Drug sensitivity

Waterfall plots showig the different treatment responses of our mesothelioma PDX towards the tested SOC chemotherapeutics and combinations. Response rates are based on treated-to-control (T/C) tumor volumes.
(> 50% T/C = resistance, 50-30% T/C = minor response, 30-5% T/C = moderate response, < 5% T/C = strong response)

Transcriptomic analyses



Unsupervised comparative genome-wide gene expression analysis based on STAR-generated RNASeq read counts normalized by variance stabilizing transformation (VST) using the *DESeq2* R-package. **(A)** Principal component analysis (PCA) resulting in 3 groups possibly reflecting the 3 histologic mesothelioma subtyps (epithelioid, sarcomatoid, biphasic). PCA done by R-package *factoextra*. **(B)** Heatmap from the distance matrix with hierarchical clustering showing a similar grouping of models. Calculated by Euclidian distance and complete linkage; generated by *pheatmap* R-package.



OncoPrint of 162 expressed protein-changing mutations in 142 cancer-related genes detected by RNASeq variant calling including 6 truncating mutations in putative cancer driver genes. 3 of them are already druggable.
Samples are arranged as follows: **Meso7212 | Meso12962 | Meso13852B | Meso14275 | Meso14731 | Meso14767**
Graphic generated by *OncoPrinter* (cBioPortal.org, Gao *et al.* SciSignal 2013 & Cerami *et al.* CancerDiscovery 2012)

Conclusion

Our established set of mesothelioma PDX will serve as a valuable preclinical tool to investigate molecular mechanisms of drug sensitivity and resistance. The presence of certain deleterious (driver) mutations that are potentially drugable underlines its potential to provide a source for translational research and for preclinical testing of new drug candidates.

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