associated with human oral squamous cell carcinomas (OSCCs). Here, we show that restoration of DOC1 expression in OSCCs leads to a reversal of Epithelial-mesenchymal transition (EMT).

**Material and methods** We examined DOC1 expression in normal and cancerous tongue tissue with immunohistochemistry. Besides, 4 different human OSCC cell lines were examined using immunofluorescence and immunoblotting. To study its role in these cells, we re-expressed DOC1 and used shRNA-mediated knockdowns (remodelers, EMT regulators). Subsequently we carried out proteomics, genomics, cell-based and biochemistry assays.

**Results and discussions** The loss of DOC1 in oral cancer cells leads to a failure of NURD to bind and repress master transcriptional regulators of EMT. Re-expression of DOC1 in OSCC cells restores NURD recruitment to key target genes, a switch from open to closed chromatin, transcriptional repression, and reversal of EMT (MET). We speculate that, during the development of oral cancer, DOC1 is lost after the inactivation of p53 and INK4a. Our genome-wide binding site analysis showed that DOC1 is crucial for NURD recruitment to a subset of target loci; in particular, promoter regions harbouring CpG islands. DOC1-mediated NURD binding to the Twist1/2 and Zeb2 promoters leads to eviction of SWI/SNF involving nucleosome repositioning, epigenetic reprogramming, and shutdown of transcription. Binding of either SWI/SNF or NURD determines opposite epigenetic states, thereby committing OSCC cells to either EMT or MET.

**Conclusion** Re-expression of DOC1 in OSCC cells restores the switch from open- to closed chromatin and reversal of EMT. We suggest that subunit-dependent gene selection is a major cause of the association between the loss of specific remodeler subunits and particular types of cancer. Our results emphasise that gene control involves a dynamic equilibrium between opposing chromatin modulating enzymes rather than a static chromatin state. Disturbances in this balance can initiate a cascade of chromatin reprogramming events that drives oncogenesis.

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**Symposium: Models for Immunotherapy**

**13** **EXPLORING MECHANISMS OF TUMOUR LYMPHANGIOGENESIS TO POTENTIATE IMMUNOTHERAPIES IN MELANOMA**

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**Introduction** Tumour lymphangiogenesis correlates with poor prognosis and metastasis in melanoma and other cancers, yet its functional roles in regulating antimelanoma immunity have remained largely unexplored. Recently, we showed that despite its pro-metastatic effects, tumour lymphangiogenesis potentiates immunotherapies in melanoma. Here, we sought to further explore mechanisms of potentiation of immunotherapies and develop means to exploit the lymphatic growth factor VEGF-C therapeutically.

**Material and methods** For this study, we used two mouse melanoma models: the injected B16F10 and the BrafV600E-PTEN\(^+\) (±\(\beta\)-CAT-STA) autochthonous models. Lymphangiogenesis was inhibited using VEGFR-3 blocking antibodies, or promoted through either viral transduction of B16F10 with VEGF-C or intratumoral injections of a matrix-binding form of VEGF-C. Immunotherapies included PD-1 blockade, immune adjuvants (CpG-B and Poly I: C), or adoptive T cell transfer. For mechanistic studies, we used Batf3\(^{-/-}\) mice, which lack cross-presenting dendritic cells (DCs) and fail to mount a potent antitumor CD8\(^+\) T cell response. Moreover, using gene expression analysis from The Cancer Genome Atlas (TCGA) database, we correlated VEGFC expression with the expression of genes associated with cross-presenting DCs such as BATF3, IRF8 and ZBTB46.

**Results and discussions** In addition to reducing T cell infiltrates, VEGF-C blockade in both models of melanoma reduced the number of cross-presenting DCs. Moreover, in tumours with low T cell infiltrates (cold tumours), and thus unresponsive to immunotherapies, the intratumoral delivery of matrix-binding VEGF-C increased T cell infiltration and restored the ability to respond to immunotherapies. Lymphangiogenic potentiation of immunotherapies was not observed in Batf3\(^{-/-}\) mice, indicating the importance of cross-presenting dendritic cells and subsequent generation of tumor-specific endogenous CD8\(^+\) T cells. Finally, in the TCGA database, we found that VEGFC expression correlated with genes associated with cross-presenting DCs in human primary melanomas.

**Conclusion** Our findings indicate that tumour lymphangiogenesis promotes recruitment of T cells and DCs into the microenvironment. By using an engineered variant of VEGF-C, we were able to transform the tumour microenvironment from a cold one to a hot one, thereby restoring responsiveness to immunotherapies. Further studies are needed to further develop this therapeutic approach.
tumour cells. In addition, AT1412 also strongly reacted with many solid tumours types including colon, pancreas and, breast cancer, as well as liquid tumours such as AML, B-ALL and multiple myeloma. Further analysis revealed that AT1412 favours binding to a clustered form of CD9. These clusters are dependent on the palmitoylation state of CD9 and known to be enriched on the surface of tumorigenic cells.

Using immunodeficient mice harbouring a human immune system (HIS mice) we observed that AT1412 blocks tumour progression as a single agent and more strongly in combination with an anti-PD1 antibody. Highly significant, AT1412 strongly stimulated the influx of macrophages and CD8+ T cells into the tumour providing an explanation for the synergistic effects of anti PD1 and AT1412.

In sharp contrast to previously described anti-CD9 antibodies AT1412 does not induce aggregation of platelets. This is in line with an absence of antibody-related adverse events, including thrombosis and colitis, in the patient during and after treatment. Altogether this indicates that the antibody is safe for use in humans.

Conclusion These data suggest that antibody AT1412 contributed to the success of the immunotherapy in this patient. This antibody could provide help to tumor-reactive immune cells in the eradication of tumour masses and is as such an attractive candidate drug for cancer treatment.

Our results show that the B-cell repertoire of a patient who is cured after immunotherapy provides a highly attractive source of antibodies with clinical potential.

Symposium: The Ras Pathway and Cancer

15 IDENTIFICATION OF NEW COMBINATION THERAPIES FOR LUNG CANCER TUMOURS HARBOURING KRAS MUTATIONS

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Introduction Oncogenic mutations in KRAS are frequent in non-small cell lung cancer (NSCLC) and have been associated with poor prognosis and resistance to existing therapies. We have previously shown that NSCLC cells harbouring KRAS mutations are more sensitive than their wild-type counterparts to inhibition of KRAS downstream effectors MEK and RAF and to treatment with IGF1R inhibitors.

Material and methods In order to identify complementary targets for the improvement of IGF1R and/or MEK targeting therapies, we performed a whole-genome shRNA screen with KRAS-mutant NSCLC cells. Validation was performed using shRNAs and small molecule inhibitors in a panel of NSCLC cell lines. In vivo efficacy of drug combinations was measured in mouse models of KRAS-induced NSCLC using CT scanning.

Results and discussions The list of sensitizers to IGF1R inhibitors included several genes encoding components of the mTOR pathway. Viability assays in a panel of lung cancer cell lines confirmed that combining IGF1R inhibitors with mTOR inhibitors, both rapalogs and kinase inhibitors, resulted in a synergistic anti-proliferative effect in KRAS-mutant NSCLC cells. Mechanistic investigations demonstrated that IGF1R inhibitors blocked reactivation of the PI3K pathway induced by mTOR inhibition, resulting in a robust suppression of PI3K and mTORC1 signalling. Addition of a MEK inhibitor to the combination produced a more profound and durable suppression of cell proliferation and a stronger induction of apoptosis by inhibiting the main downstream pathways controlled by KRAS. Notably, the inhibition of these signalling pathways was stronger in KRAS-mutant cells than in wild-type cells. In order to achieve strong downstream pathway inhibition even more specifically in KRAS-mutant cells, we used the KRAS-G12C mutant inhibitor, ARS-1620. Interestingly, addition of mTOR and IGF1R inhibitors vastly increases effectiveness of the KRAS-G12C inhibitor.

Finally, we validated the drug combinations in mouse models of KRAS-induced NSCLC. Results showed that combined mTOR, IGF1R and MEK inhibition produced a marked tumour regression in a NSCLC mouse model driven by mutant Kras and p53 loss-of-function and also in urethane-induced lung tumours.

Conclusion We have demonstrated that a profound inhibition of the main pathways downstream KRAS is needed to achieve a durable suppression of cell viability in NSCLC cells harbouring KRAS mutations. These findings suggest potential novel therapeutic strategies for KRAS mutant NSCLC tumours.

16 QUADRUPLE VERTICAL TARGETING OF AN ONCOGENIC PATHWAY AS A TREATMENT STRATEGY TO PREVENT DRUG RESISTANCE

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Introduction Targeted cancer drugs elicit powerful initial responses but far less long-term benefits. The emergence of therapy resistance often involves the reactivation of the same pathway that is being blocked by the cancer drug. Our group and others have shown that inhibiting multiple nodes of the same activated signalling pathway provides longer-lasting therapeutic benefits. This ‘vertical’ inhibition strategy is also used by microRNAs to efficiently ‘silence’ a signalling pathway by partially inhibiting multiple nodes of the same pathway.

Material and methods We tested this concept in an EGFR-driven NSCLC model. PC9 cells were treated with low concentrations (~IC10–20) of Gefitinib, LY3009120, Trametinib and SCH772984, which inhibit EGFR, RAF, MEK and ERK, respectively. We then measured the effect on pathway inhibition, on cell viability and on the development of resistance.

Results and discussions Using this strategy, we were able to fully block the MAPK pathway, which resulted in abrogation of cell proliferation and induction of apoptosis in PC9 cells. We then expanded our study to EML4-ALK-driven NSCLC, EGFR-driven CRC and HER2-driven breast cancer, having successfully validated the strategy in all these settings. Also, using mouse xenograft models, we have shown that this strategy induces a quick and durable tumour control, without associated toxicity. Further, using genome wide CRISPR/Cas9 genetic screens, we have shown that by using this concept we are able to prevent the occurrence of resistance. Our findings