Characterization of a TGFβ signaling-associated network in melanoma drug resistance and invasiveness

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Melanoma is a cancer typically occurring in the skin. Its most effective treatment is surgery; however, this is not possible when it has metastasized. Available drug treatments in that situation target BRAF or use immunotherapeutic approaches but have very limited success. Despite the TGFβ pathway usually inhibiting cancer growth, in melanoma cells it has recently been shown (Spender et al, 2016) that mutant BRAFV600E can lead to TGFβ pathway dependency. The TGFβ pathway has also been found to be implicated in melanoma cells that show resistance to treatment (Huang et al, 2012) and to lead to epithelial-to-mesenchymal transition and promote metastasis (Perrot et al, 2013).

The aims of this project are:
a) Characterization and modelling of context-specific TGFβ signalling cross-talk with other pathways in melanoma and during its treatment.
b) Discovery of genes conferring resistance to current targeted melanoma treatments and identification of potential combination targets in the TGFβ signalling-associated network.
c) Discovery of genes affecting the TGFβ signalling-associated network and cause changes in cell morphology as potential markers for metastasis.

Methodology

A. CRISPR Screening and data collection (Wright lab)
The SK-MEL-1 cell line is derived from human metastatic malignant melanoma and is positive for the BRAFV600E mutation. The Wright lab has generated SK-MEL-1 cell lines stably expressing Cas9. The successful ESPOD fellow will use this cell line to create initially 6 different signaling reporter cell lines (No reporter, TGFβ, MAPK, NFkB, PI3K/AKT and Wnt) using the Cignal[TM] GFP reporters from Qiagen. These reporters have been selected because they are all known to cross-talk wit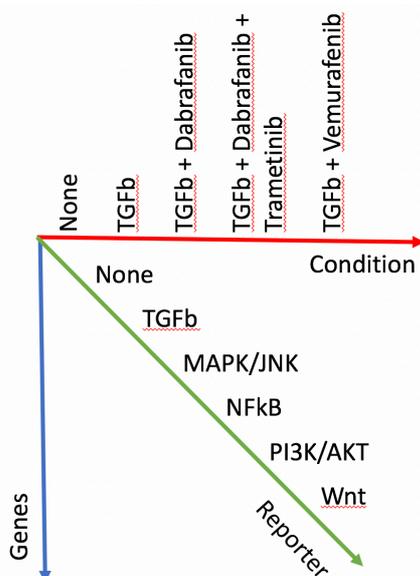h TGFβ signalling (Guo and Wang, 2009) and will thus be activated by stimulation with TGFβ. After appropriate validation of functional reporter cell lines, whole-genome CRISPR will be performed to create 6 distinct reporter gene knockout libraries. These libraries will then be treated with known melanoma drugs (BRAF and MEK inhibitors; Figure 1) in the presence of TGFβ and sorted using FACS. A subset of the identified hits will be confirmed individually as a validation of the screens.

Figure 1. Matrix of conditions to test

B. Data analysis (Petsalaki lab)
After initial validation of the experimental outcome based on existing knowledge of the tested signaling pathways, the candidate will initially perform an exploratory analysis by mapping the hits on a network comprising the components and interaction networks

of the pathways assayed that are already annotated as being involved in the TGFβ signalling network. Outliers and unexpected hits will be identified as potential novel candidates involved in this network.

As a next step the ESPOD fellow will create a model of the signal propagation in melanoma cells in the tested conditions. Integrating existing gene expression and genomic data for this cell line from the COSMIC database Cell Lines project with available proteomics data and phosphoproteomics data from melanoma cell lines (e.g. Parker et al, 2015), the ESPOD fellow will initially use different statistical approaches (e.g. Hill et al 2016) to reconstruct the expected signaling networks active during treatment of melanoma cells with the tested drugs. The novel hits identified in the screen will be mapped on the network along with their effect on the signaling pathways tested. By combining these data and prior knowledge, a TGFβ signalling-associated network will be defined. The fellow will then use existing approaches (e.g. Halasz et al, 2016 or Paull et al, 2013) and also others developed in the Petsalaki lab, to model the signal propagation through this network and predict the outcome of *in silico* perturbations on the network and signal activation. We expect that this model will highlight the cross-talk between TGFβ and the different pathways and functional modules associated in this context. The ESPOD fellow will then use the model to identify potential genetic interactions that can be used for combination therapy to overcome drug resistance in the cell line, to induce normal TGFβ response leading to cell cycle arrest, or to prioritize candidates that have a strong effect on the network for follow-up experiments to better understand the role of the TGFβ signalling-associated network in melanoma invasiveness and metastasis.

C. Follow up experiments (in collaboration with Bakal lab, ICR)

Given the role of TGFβ signalling in melanoma EMT and metastasis, we will collaborate with the Bakal lab at the ICR in London, to associate the predicted and measured effects of gene knock-out on the TGFβ signalling network with effects on cell morphology that could have an impact on the metastatic potential. This will be achieved through high-content live single-cell imaging in the different conditions we tested on gene hits that will be prioritized for this study using the model created above. A subset of genes will be selected for further migration studies e.g. through a scratch assay or in 3D cultures. Using the signalling model described above the post doc will attempt to identify gene targets to inhibit metastasis and if possible the predictions will be tested through either double gene knock-out and/or drug application.

 Expected outcome

Through this project the ESPOD fellow will discover novel genes that mediate resistance to current treatment of malignant melanoma and will uncover the molecular mechanism underlying this resistance in order to rationally identify potential targets to overcome it. They will moreover create an executable model of TGFβ signalling and its cross-talk with other major signalling pathways, that can be further used as a starting point to model this functional network in other healthy or disease conditions. Finally, this project has the potential to identify TGFβ-signalling related invasiveness or metastasis biomarkers for melanoma, which could aid the discovery of drugs to limit the metastatic potential of the disease, allowing the extension of the time-frame during which it can be treated through surgery, which currently is the most effective treatment.