Non-Canonical Hedgehog Signaling is a Positive Regulator of the WNT Pathway and is Required for the Survival of Colon Cancer Stem Cells

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CHALLENGE
Color cancer is a heterogeneous tumor that represents the third most common type of cancer and first most common cause of cancer deaths worldwide. Current evidence supports the existence of a subpopulation of cancer stem cells (CSCs), as both the drivers of tumor growth and the source of repopulation following treatment. Inactivation of the molecular pathways that regulate CSC survival and contribute to tumor heterogeneity may therefore lead to more effective treatments. (Figure 1)

RESULTS
Conventional cancer therapy and targeted CSC therapy
Wnt and hedgehog signaling frequently cooperate to control cell fate, homeostasis and cancer. In the intestine, Wnt signaling drives crypt-base stem cell turnover. Commonly, GLI-independent Hedgehog signaling antagonizes Wnt signaling in differentiated cells at the base of the crypt. Activating mutations in Wnt signaling are found in 85% of colon cancers. Hedgehog signaling, while rarely mutated, is upregulated in colon cancer. However, therapeutic strategies that directly target Wnt or canonical (SMO-dependent) Hedgehog signaling have been unsuccessful. Here, as part of the Oncotrack consortium, we used patient-derived organoids (PDOs) and xenograft models of colon cancer to demonstrate the survival of colon CSCs is dependent on non-canonical (SMO-independent) Hedgehog signaling, which acts as a positive regulator of Wnt signaling regulating CSC differentiation and survival. (Figure 2)

THE ONCOTRACK CONSORTIUM
Goal: To develop and assess novel approaches for the identification of new biomarkers for colon cancer
Approach: Detailed characterization of colon cancer cells combining novel in vitro and in vivo models, high-throughput sequencing and systems biology
Start date: 01.01.2011
IMI funding: €16.757.282
EFPIA in kind: €1.976.557
Other: € 3.346.480
www.oncotrack.eu
Total cost: € 31.080.319

APPROACH & METHODOLOGY
PDOs were generated in Neuro2A culture from freshly isolated primary tumors and metastases. The frequency of CSCs was determined by transplantation (Figure 2A). Increased cell proliferation (ADLI; Figure 2A) and increased cell number (Figure 2B) were observed in colon CSCs. (Figure 2B) ALDHI+AluC* cells were subjected to whole-genome analysis (Figure 2C). ALDHI+AluC* cells were subjected to functional analysis by RNA interference and small-molecule inhibition

Figure 1. Conventional cancer therapy and targeted CSC therapy

Conventional cancer therapy
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Figure 2. Summary of study methodology

Figure 3. CSCs in colon cancer PDOs vary in frequency and are enriched in more advanced tumors. (A) LD-transplantation of PDO cells. Growth curves (B) and EMT (C) in line a5 xenograft models.

Figure 4. CSC-Enriched PDOs are heterogeneous, poorly differentiated and enriched for Wnt signaling genes. (A) Immuno-histochemical staining of PDOs. (B) Heatmap showing enrichment for Wnt signaling genes.

Figure 5. PDOs are enriched for ALDH1A1, CSCs and non-enriched for FACS plots. (B) Frequency of ALDH1A1 cells in PDOs and xenografts. (C) LD-serial transplantation table. (D) EMT and (E) gene expression analysis of ALDH1A1- and ALDHI- CSCs in the xenografts. Immunohistochemical staining of PDOs (F) and DTC (C) 'tumor-like' structures in formalin-fixed xenografts.

Figure 6. Non-canonical PTC1H1-dependent Hedgehog signaling is a positive regulator of Wnt signaling (A) PDO survival 72 h after treatment with Tankyrase inhibitor, SMO inhibitors vismodegib and cyclopamine, and the A2780 xenograft PTC1H1 inhibitor R1545.33 (B) PDO survival with Wnt signaling reporter PDOs. (C) Abnormality of FACS plots. (D) TCF/LEF eGFP-gene expression analysis. FACS plots (E and F) of TCR1 and FGF10-treated PDOs with vismodegib or U-0126.

Figure 7. Non-canonical Hedgehog signaling is required for CSC survival and regulates differentiation in vivo. (A) EDNRB knockout mice. (B and C) Deficiency of EDNRB: HHAT on sphere formation. (D) LD-1 xenograft HHAT transplants. (E) FGF10 induction and growth curves of EDNRB HHAT xenografts. (F) Gene expression analysis of EDNRB HHAT xenografts. (G) Possible correlation of Hedgehog genes in clinical samples. (H) PTC1H1 expression in colon cancer patients across different tumor stages.

Figure 8. Relationship between Hedgehog and Wnt signaling in the regulation of colon CSC differentiation.

CONCLUSIONS & IMPACT
Colorectal cancer PDOs are enriched for CSCs and Wnt signaling genes. Hedgehog signaling in CSCs is non-canonical and PTC1H1-dependent. Non-canonical hedgehog signaling is a positive regulator of Wnt signaling (Figure 8). CSC survival depends on HHAT-mediated proliferation of EDNRB HHAT as a possible therapeutic target. PTC1H1 is a potential biomarker for colon cancer prognosis.

VALUE OF IMI COLLABORATION
- Patient recruitment and tissue collection by public partners
- Access to novel patient-derived tumor models, primary tissue samples, sequencing and clinical data from public and private consortium partners
- Access to small molecule inhibitors from EFPIA partners
- Establishment of robust and sustainable business models
- Development of innovative solutions for the continuing challenges in cancer research

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