KRAS oncogenes have been identified in at least one fifth of all human cancers. In spite of recent successes with checkpoint inhibitors, most KRAS mutant tumours, including lung adenocarcinomas, are still treated with cytotoxic compounds approved over 2 decades ago. Moreover, attempts to block KRAS oncogenic activity with selective inhibitors of the MEK kinase, a downstream effector, have turned out to be major failures. Two MEK inhibitors, Trametinib and Selumetinib, have failed to show significant anti-tumour activity in large phase III clinical trials due to unacceptable toxicities. In our laboratory, we have continued our quest to validate therapeutic targets using a new generation of genetically engineered mouse tumour models that allow us to evaluate their anti-tumour properties as well as their potential toxic effects in tumour-bearing mice. These studies have allowed the identification of c-RAF as a target capable of inducing significant tumour regressions in advanced KRAS/Trp53 mutant lung tumours without inducing major toxicities. These observations suggest that forthcoming c-RAF inhibitors may provide significant therapeutic benefits in the clinic.

“Systemic ablation of c-RAF induces regression of a significant percentage of advanced K-Ras/Trp53 mutant lung adenocarcinomas by a mechanism independent of MAPK signalling that results in the induction of acceptable toxicities.”
**Regression of advanced K-Ras/Tp53 mutant lung adenocarcinomas upon systemic ablation of c-RAF expression**

Almost a quarter of all solid tumours harbour K-Ras/Tp53 oncogenes. Yet, more than 30 years after their identification in human cancer, there are no selective therapies to treat these tumours. Inhibitors of K-Ras oncogenes activated by G12C mutations, a mutation frequently identified in lung adenocarcinomas, have already entered clinical trials. Yet, direct targeting of other mutations has proven to be challenging. Genetic interrogation of the MAPK pathway revealed that systemic ablation of MEK or Erk kinases in adult mice is accompanied by a significant degree of tumour regression with no detectable appearance of resistance mechanisms (Figure 1). Tumour progression results from massive apoptosis. Importantly, systemic abrogation of c-RAF expression does not inhibit canonical MAPK signalling, hence, resulting in limited toxicities. These observations suggest that therapeutic strategies aimed at inhibiting c-RAF kinase activity may not be suitable since they may only block MAPK Kinase activation. Indeed, three independent c-RAF kinase inhibitors have shown to be rather toxic even at non-toxic doses. Therefore, we need to explore other therapeutic strategies. Drugs capable of degrading the c-RAF protein could be more effective in the clinic. Yet, drugs that promote protein degradation are still at very early stages of development. Inhibition of c-RAF by small interfering RNA is an appealing possibility, but still face significant technical challenges. Finally, selective targeting c-RAF effectors regulated through non-kinase mechanisms such as BOKapla, ASK1 and MST2 will also be challenging since they will require to be active in both tumour cells and CAFs. As a consequence, germline ablation of Saa3 does not prevent PDAC tumour development in mice (Figure 2). The pro-tumorigenic activity of Saa3 in CAFs is mediated by Mmps, a member of the p314tamorolled membrane protein subfamily of the peripheral membrane-associated guanylate kinases. Finally, we interrogated whether these observations could be translated to a human scenario. Indeed, Saa3, the orthologue of murine Saa, is overexpressed in human CAFs. Moreover, high levels of Saa3 in the stromal component correlate with worse survival. These findings support the concept that selective inhibition of Saa3 in CAFs may provide potential therapeutic benefit to PDAC patients.

---

**REFERENCES**

1. Saam M, Aigner P, Stiedl P, Hidalgo M, Penninger J, Barbacid M (2018) Saa3 is a key mediator of the pro-tumorigenic properties of cancer-associated fibroblasts in pancreatic ductal adenocarcinomas. Pancreatic ductal adenocarcinoma (PDAC) is one of the most malignant human tumours for which there are no efficacious therapeutic strategies. This tumour type is characterised by an abundant desmoplastic stroma that promotes tumour progression. It is generally accepted that cancer-associated fibroblasts (CAFs) stimulate tumour progression and might be implicated in drug resistance and immunosuppression. Yet, recent studies have shown that physical or genetic elimination of the stroma leads to more aggressive tumour development. In an attempt to clarify the role of the desmoplastic stroma in PDAC development and progression, we decided to reprogram the CAFs that make up the stromal tissue by identifying, and subsequently targeting, genes responsible for their pro-tumorigenic properties. First, we compared the transcriptional profile of PDGF-CAFs isolated from genetically engineered mouse PDAC tumours with that of normal pancreatic fibroblasts (NPFs) in order to identify genes potentially implicated in their pro-tumorigenic properties. We have observed that the most differentially expressed gene, Serpoamyloid A (Saa3) apolipoprotein family, is a key mediator of the pro-tumorigenic activity of PDGF-CAFs. Whereas Saa3 competent CAFs stimulate the growth of PDAC tumour cells in an orthotopic model, Saa3 null CAFs inhibit tumour growth. Saa3 plays a role in the cross-talk between CAFs and tumour cells (FIGURE 2). Ablation of Saa3 in pancreatic tumour cells makes them insensitive to the inhibitory effect of Saa3 null CAFs. As a consequence, germline ablation of Saa3 does not prevent PDAC tumour development in mice (FIGURE 2). The pro-tumorigenic activity of Saa3 in CAFs is mediated by Mmps, a member of the p314tamorolled membrane protein subfamily of the peripheral membrane-associated guanylate kinases. Finally, we interrogated whether these observations could be translated to a human scenario. Indeed, Saa3, the orthologue of murine Saa, is overexpressed in human CAFs. Moreover, high levels of Saa3 in the stromal component correlate with worse survival. These findings support the concept that selective inhibition of Saa3 in CAFs may provide potential therapeutic benefit to PDAC patients.

---

**Figure 1** Systemic ablation of c-RAF expression results in equal or better levels of tumour regression while inducing acceptable toxicities. In contrast, inhibition of the MAPK kinase pathway induces tumour regression but with unacceptable toxicities both in mice (Blasco et al., Cancer Cell 2017) and in patients (Ganem and Selhamer submitted clinical trials).

**Figure 2** Crosslink between pancreatic tumour cells and CAFs in the presence and absence of Saa3. Diagram depicting the in vivo orthotopic tumour assay in immunodeficient mice carried out to determine the pro-tumorigenic properties of Saa3 competent (WT) and Saa3 null (KO) (light blue) CAFs on pancreatic tumour cells isolated from Saa3 competent (WT) (yellow) and Saa3 null (KO) (green) tumours.

---

**CONCLUSION**

Pancreatic ductal adenocarcinoma (PDAC) is one of the most malignant human tumours for which there are no efficacious therapeutic strategies. This tumour type is characterised by an abundant desmoplastic stroma that promotes tumour progression. It is generally accepted that cancer-associated fibroblasts (CAFs) stimulate tumour progression and might be implicated in drug resistance and immunosuppression. Yet, recent studies have shown that physical or genetic elimination of the stroma leads to more aggressive tumour development. In an attempt to clarify the role of the desmoplastic stroma in PDAC development and progression, we decided to reprogram the CAFs that make up the stromal tissue by identifying, and subsequently targeting, genes responsible for their pro-tumorigenic properties. First, we compared the transcriptional profile of PDGF-CAFs isolated from genetically engineered mouse PDAC tumours with that of normal pancreatic fibroblasts (NPFs) in order to identify genes potentially implicated in their pro-tumorigenic properties. We have observed that the most differentially expressed gene, Serpoamyloid A (Saa3) apolipoprotein family, is a key mediator of the pro-tumorigenic activity of PDGF-CAFs. Whereas Saa3 competent CAFs stimulate the growth of PDAC tumour cells in an orthotopic model, Saa3 null CAFs inhibit tumour growth. Saa3 plays a role in the cross-talk between CAFs and tumour cells (FIGURE 2). Ablation of Saa3 in pancreatic tumour cells makes them insensitive to the inhibitory effect of Saa3 null CAFs. As a consequence, germline ablation of Saa3 does not prevent PDAC tumour development in mice (FIGURE 2). The pro-tumorigenic activity of Saa3 in CAFs is mediated by Mmps, a member of the p314tamorolled membrane protein subfamily of the peripheral membrane-associated guanylate kinases. Finally, we interrogated whether these observations could be translated to a human scenario. Indeed, Saa3, the orthologue of murine Saa, is overexpressed in human CAFs. Moreover, high levels of Saa3 in the stromal component correlate with worse survival. These findings support the concept that selective inhibition of Saa3 in CAFs may provide potential therapeutic benefit to PDAC patients.

---

**Figure 1** Systemic ablation of c-RAF expression results in equal or better levels of tumour regression while inducing acceptable toxicities. In contrast, inhibition of the MAPK kinase pathway induces tumour regression but with unacceptable toxicities both in mice (Blasco et al., Cancer Cell 2017) and in patients (Ganem and Selhamer submitted clinical trials).

**Figure 2** Crosslink between pancreatic tumour cells and CAFs in the presence and absence of Saa3. Diagram depicting the in vivo orthotopic tumour assay in immunodeficient mice carried out to determine the pro-tumorigenic properties of Saa3 competent (WT) and Saa3 null (KO) (light blue) CAFs on pancreatic tumour cells isolated from Saa3 competent (WT) (yellow) and Saa3 null (KO) (green) tumours.