TUMOUR SUPPRESSORS

mRNA alterations mimic genetic mutations

Credit: Lara Crow/Springer Nature Limited

Intronic polyadenylation (IPA) of mRNAs occurs in immune cells to generate transcriptome diversity. Given that RNA processing (specifically splicing) is altered in chronic lymphocytic leukaemia (CLL), and that many CLLs carry <2 somatic mutations in known driver genes, Lee, Singh et al. hypothesized that cancer-specific IPA events might generate truncated proteins that could act as CLL drivers.

Using primary samples from patients and healthy donors, the authors identified and validated nearly 5,000 IPA isoforms, ~600 of which had higher expression in CLL compared with normal B cells (CLL-IPAs). Of these, 330 CLL-IPAs, affecting 306 genes, were recurrent (occurred in >10% of the CLL samples).

Analysis of 13 candidate CLL-IPAs indicated that they produced stably expressed truncated proteins that are predicted to be similar to those produced by truncating somatic mutations. This suggests that IPAs could potentially inactivate tumour suppressor genes (TSGs). Indeed, many of the genes affected by CLL-IPAs were TSGs. Importantly, TSGs inactivated by known truncating somatic mutations in CLL were enriched among CLL-IPAs, indicating that CLL-IPAs could be an alternative cancer driving mechanism.

Further study of several of the candidate CLL-IPAs indicated that at least some functionally mimic genetic mutations previously observed in cancer.

For example, CARD11, a positive regulator of the nuclear factor-κB (NF-κB) pathway, is affected by a CLL-IPA. The CARD11 IPA results in a truncated protein that is more stable than wild-type CARD11 and more potently activates NF-κB. This may mimic the effects of *CARD11* activating mutations, which are observed in another B cell malignancy, diffuse large B cell lymphoma.

As another example, an IPA in DICER abrogated the ability of the protein to cleave microRNAs. The truncated DICER IPA protein mimics truncating mutations of *DICER* found in Wilms tumours.

Similarly, the TSG *MGA*, which normally antagonizes MYC transcriptional programmes, is affected by truncating mutations in CLL and solid tumours.

> “CLL-IPAs could be an alternative cancer driving mechanism”

TUMOUR MODIFIERS

STAT3’s true colours

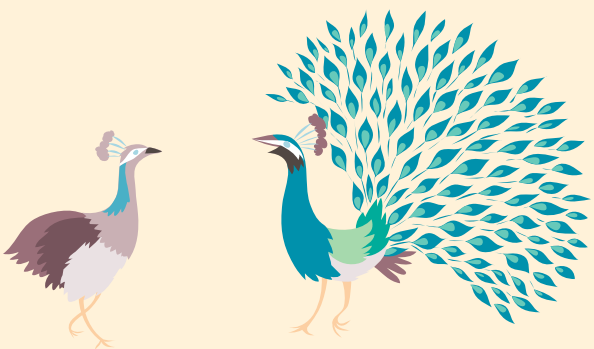
Signal transducer and activator of transcription 3 (STAT3) has been described to have contrasting roles in tumour development, suggesting a context-dependent action. A study now shows that, in the presence of oncogenic RAS mutations, STAT3 acts as a tumour modifier by regulating the epithelial differentiation of pancreatic and lung cancer cells via p63.

STAT3 is activated in response to cytokines and growth factors via Janus kinase (JAK)-mediated phosphorylation of Tyr705 (Y705). D’Amico et al. initially observed, by querying >800 samples in The Cancer Genome Atlas database, that in pancreatic ductal adenocarcinoma (PDAC), lung adenocarcinoma (ADC) and lung squamous cell carcinoma (SCC), there is little alteration of phosphorylated Tyr705 (pY705). They further showed that, while upregulation of STAT3 and pY705 levels is common in early pancreatic intraepithelial lesions, most PDAC samples lose STAT3 expression and only a few of the cases that express STAT3 show pY705 staining. This suggests a possible tumour-suppressive action of STAT3 at late stages of cancer development.

> “highlighting the importance of cell context for STAT3 function”

To further dissect the role of STAT3 in pancreatic cancer, the authors utilized a PDAC mouse model with $Kras^{G12D}$ expression and *Trp53* homozygous deletion. They isolated premalignant pancreatic cancer cells from the mice and modified the cells ex vivo by either knocking out STAT3 or by overexpressing wild-type or hyperactive STAT3 mutants. Loss of STAT3 reduced the expression of differentiation markers and increased mesenchymal markers corresponding to a consistent histology of anaplastic PDAC when transplanted either subcutaneously or orthotopically into nude mice. On the contrary, the expression of STAT3 or hyperactive STAT3 resulted in an increase of differentiation markers in vitro, and a marked delay in tumour onset and a clear histology of well-differentiated tumours in vivo. They confirmed these results in another epithelial cell type by applying the same strategy to cells isolated from the lungs of adult $Kras^{G12D}$;$Trp53^{-/-}$ mice. In these cells, STAT3 deletion promoted undifferentiated carcinosarcomas while STAT3 expression or activation delayed tumour onset and skewed the phenotype of the tumours towards well-differentiated SCC.

STAT3 activation in the lung model increased levels of p63, a critical protein for epithelial development.



Credit: Lara Crow/Springer Nature Limited

The MGA IPA observed in CLL acts as a dominant negative, promoting MYC-driven transcription.

An analysis of genes affected by CLL-IPAs in ≥20% of samples (190 genes) indicated that 72% have recurrent truncating mutations in solid tumours. Many of these have low somatic mutation rates and are not known TSGs, but they are candidates for future research.

It seems likely that the authors' findings are not restricted to CLL, as an IPA of the TSG *MAGI3* was previously reported to have dominant-negative activity in breast cancer. In addition, the authors found >100 IPAs that were upregulated in T cell acute lymphoblastic leukaemia (T-ALL).

Overall, this study emphasizes the importance of analysing non-genetic alterations when examining the driver events responsible for a given tumour.

Sarah Seton-Rogers

ORIGINAL ARTICLE Lee, S. H., Singh, I. et al. Widespread intronic polyadenylation inactivates tumour suppressor genes in leukaemia. *Nature* **561**, 127–131 (2018)

p63 expression was unaltered in the pancreatic model, but the authors found that in both lung and pancreatic $Kras^{G12D}$;$Trp53^{-/-}$ cells depleted of p63, STAT3 expression or activation failed to induce differentiation. Interestingly, p63 expression alone was sufficient to induce differentiation of STAT3-depleted lung cancer cells while it had only marginal effects when tested in the equivalent pancreatic cancer setup, further highlighting the importance of cell context for STAT3 function.

These findings show an unexpected function for STAT3 in a $Kras^{G12D}$-mutated epithelial background. However, it is possible that STAT3 function might be different in the presence of different oncogenic alterations. The definition of such interplays would be important to define appropriate therapeutic interventions.

Maria Giuseppina Baratta,
Associate Editor,
Nature Communications

ORIGINAL ARTICLE D'Amico, S. et al. STAT3 is a master regulator of epithelial identity and KRAS-driven tumorigenesis. *Genes Dev.* **32**, 1175–1187 (2018)

TUMOUR MICROENVIRONMENT

Neighbourly deaths dictate fate

Credit: Lara Crow/Springer Nature Limited

Primary liver cancers consist of hepatocellular carcinomas (HCCs) and intrahepatic cholangiocarcinomas (ICCs), which differ substantially in their histology, metastatic potential and prognosis. Studies in mice have indicated that ICC and HCC can share the same cell of origin, the hepatocyte. Yet, the mechanisms regulating the diversity in the commitment of transformed hepatocytes remain largely unknown. As primary liver cancers invariably develop in chronically damaged livers, this suggests that tissue context can determine the tumour subtype identity. Now, Seehawer, Heinzmann et al. have shown that hepatocytes harbouring the same oncogenic drivers can give rise to either HCC or ICC dependent on the type of cell death occurring nearby in the liver microenvironment.

Generating in vivo models of liver cancer, the authors used two different methods to deliver transposon vectors co-expressing oncogenic mouse *Myc* and $Nras^{G12V}$ (pCaMIN) or mouse *Myc* and human *AKT1* (pCaMIA) into the livers of $p19Arf^{-/-}$ mice. When the genes were delivered by hydrodynamic tail vein injection (HDTV), the mice developed multifocal HCC with cancer cells strongly expressing the hepatocyte-specific transcription factor hepatocyte nuclear factor 4α (HNF4α). However, injection of the same vectors under the liver capsule followed by in vivo electroporation (Epo) resulted in the appearance of unilocular ICC or mixed HCC–ICC with cancer cells staining positive for biliary type keratin 19 (K19). Moreover, in vivo lineage tracing demonstrated that both tumour subtypes originated from differentiated hepatocytes.

After eliminating the possibility that spontaneously acquired genetic mutations drive the differential lineage commitment to ICC or HCC, the authors hypothesized that variation in the liver microenvironment generated by the delivery method could be important. Following either HDTV or Epo treatment, damaged liver tissue with a concomitant inflammatory reaction could be observed, with similar numbers of hepatic stellate cells and infiltrating inflammatory and immune cells. Interestingly, while the number of dying hepatocytes was also equal between the two treatments, the type of cell death induced was different. Expression of the apoptosis marker cleaved caspase 3 was higher after HDTV whereas Epo-treated livers exhibited increased levels of phosphorylated MLKL and RIPK3, both markers of necroptotic cell death.

Necroptosis is typically associated with release of inflammatory cytokines from immune cells. Concordant with this, a change in the cytokine microenvironment was observed when mouse livers were subjected to Epo-treatment versus HDTV. To establish whether the necroptotic cell death occurring in Epo-treated livers was responsible for the increased induction of cytokines, such as CCL4 and CXCL13, mice were pre-treated with necrostatin 1, an inhibitor of necroptosis before Epo. This resulted in a decrease in the production of most Epo-related cytokines as well as a switch towards apoptotic cell death and the outgrowth of $HNF4\alpha^{+}$ HCCs.

Reasoning that the fate of transformed hepatocytes in liver tumorigenesis might be epigenetically controlled, the authors identified two transcription factors, TBX3 and PRDM5, as potential lineage commitment factors with reciprocal patterns of expression in HCC and ICC. Remarkably, HDTV delivery of pCaMIN vectors co-expressing full-length *Prdm5* together with a *Tbx3* short hairpin RNA switched the outgrowth of HCC towards the development of $HNF4\alpha^{-}$;$K19^{+}$ ICCs.

This mechanism of lineage determination seems to be conserved in humans as analysis of 199 tumour samples revealed a necroptosis gene signature associated with patients with ICC and an apoptosis gene signature enriched in patients with HCC. Likewise, the gene expression patterns of *TBX3* and *PRDM3* in human HCC and ICC recapitulated the mouse models.

Not only does this study provide a molecular basis for why common liver-damaging risk factors predispose to either HCC or ICC, but it also establishes valuable mouse models to investigate these early events in tumorigenesis.

Anna Dart

ORIGINAL ARTICLE Seehawer, M. & Heinzmann, F. et al. Necroptosis microenvironment determines lineage commitment in liver cancer. *Nature* https://doi.org/10.1038/s41586-018-0519-y (2018)