**Supplementary Discussion**

Similar to *Drosophila* germ cells, mouse embryonic and hematopoietic stem cells\(^1\)\(^-\)\(^4\), also epidermal bulge stem cells switch from low to high protein translation rates when undergoing lineage commitment. Notably, this increase in protein translation is determined by lineage commitment but not cell proliferation. This finding is unexpected because a general increase in protein synthesis by perturbation of the translational machinery has been linked to increased cancer susceptibility\(^5\).

Elevated protein synthesis and increased cell division rates are key physiological tasks for cancer cells and targeting protein translation has emerged as a promising therapeutic tool to control tumour onset and progression\(^5\)\(^-\)\(^7\). However, the heterogeneity of tumours makes it difficult to predict how protein translation is regulated in distinct cell populations. We find that, similar to normal epidermal stem cells, also tumour-initiating cells in squamous tumours produced less protein than their immediate progenitors in the suprabasal layers. We propose that up-regulation of protein synthesis promotes differentiation because inhibition of protein translation rates increased tumour-initiating cell self-renewal and delayed differentiation.

We find that methylation of transfer RNAs by NSUN2 supports a boost of global translation in committed progenitors in squamous tumours. NSUN2 was originally identified as a direct downstream target gene of MYC and is up-regulated in epithelial tumours\(^8\)\(^,\)\(^9\). MYC is a pleiotropic transcription factor that also coordinates protein synthesis through the transcriptional control of RNA and protein components of ribosomes and translation initiation factors\(^10\)\(^,\)\(^11\). Thus, it is intriguing to speculate that MYC requires NSUN2 for its function in regulating protein synthesis.

In line with our previous finding that epidermal proliferation was unaffected in *Nsun2* knockout mice\(^12\), we now reveal that NSUN2-mediated RNA methylation in squamous tumours does not determine proliferation rates but is essential for tumour-initiating cells to undergo differentiation. Although our previous studies showed that knockdown of *Nsun2* in MYC-over-expressing keratinocytes reduced cell division rate, proliferation in a wide range of cultured human tumour cell lines, including human squamous cell carcinomas was unaltered in the absence of NSUN2\(^8\)\(^,\)\(^9\). However, growth of human squamous cell carcinoma xenografts into nude mice was decreased when expression of NSUN2 was inhibited by RNAi\(^8\). We now also identify NSUN2 as an important mediator for tumour cell survival under stress, and the failure of the human tumour cells to engraft into the mouse environment might rather reflect their inability to activate survival pathways than impaired proliferation.

Similar to previous findings, we now show that m\(^5\)C methylation of tRNAs protects them from endonucleolytic cleavage and deletion of NSUN2 induces 5’ tRNA fragments accumulation in tumours\(^13\)\(^,\)\(^14\). Whether 5’ tRNA fragments inhibit translation globally or transcript-specific was unknown. Our ribosome profiling study in squamous tumours revealed that inhibition of global protein synthesis in *Nsun2/-* cells is accompanied by an increase of ribosomes in 5’ UTRs of
distinct groups of genes involved in apoptosis, stress response and cell motility. How ribosomal footprint increase in these specific 5’ UTR affects protein production remains unclear. While translation of up-stream open reading frames in 5’ UTR represses translation of some mRNAs others permit downstream re-initiation\textsuperscript{15-17}. A recent study showed that re-initiation of distinct classes of mRNAs can occur independently of canonical initiation via the DNR-MCT-I (DNR) complex in \textit{Drosophila}\textsuperscript{18}. Notably, DNR-mediated translation initiation was not required for quiescent cells but needed for the exit of quiescence\textsuperscript{18}. Similarly, we find that the specific translational programme that is evoked in the absence of NSUN2 is required to maintain stem cell functions. Furthermore, we reveal that this translational programme must be revoked in stem cells not only to allow cell differentiation but also to survive in response to cytotoxic drugs.

Our data reveal that the sensitivity of \textit{Nsun2-/-} cells to cytotoxic drugs directly depends on angiogenin-mediated tRNA cleavage. Alterations in the translation apparatus through oncogenic modulation of translation initiation factors, tRNA modifying enzymes or tRNA levels are often linked to cancer\textsuperscript{19,20}. Translational control is a crucial component of cancer development and progression and steers specific cancer cell behaviours including cell survival, angiogenesis, metastasis and invasion through selective translation of the respective mRNAs\textsuperscript{7,19}. Notably, our ribosome profiling approach identified specific translated mRNAs from the same categories: cell migration, angiogenesis and survival, as candidates to promote stem cell function and tumourigenesis.

Our finding that RNA methylases play a substantial role in tumour regeneration after chemotherapeutic treatment may further suggest a combinatorial treatment of RNA methylase inhibitor plus chemotherapeutic agents as effective anti-cancer treatment. In conclusion, our data reveal a novel mechanism for controlling intrinsic cancer cell behaviours that is uniquely dependent on translational regulation.

\textbf{References}


