of culture, in agreement with previous observations[6].

The identification of SC-EC was surprising, because enterochromaffin cells are not normally present in the pancreas. Notably, these cells remained stable for up to eight weeks after transplantation into mice. The ratio of SC-EC to pancreatic endocrine cells (SC-α and SC-β) varied substantially when the authors changed the cocktail of signalling factors given to the cells at the differentiation stage that matches the induction and patterning of the gut and pancreas *in vivo*. This observation suggests that the specification of pancreatic and gut progenitor cells is essential for the generation of organ-specific endocrine cells. Different combinations of signalling factors also produced different ratios of non-proliferative endocrine cell types and proliferative non-endocrine cell types (acinar and ductal pancreatic cells).

To engineer safe and functionally mature stem-cell-derived islet equivalents, the desired endocrine-cell subtypes must be isolated and purified, and then reaggregated into pseudoislets[4]. The authors found that a simple dissociation and reaggregation procedure can remove most of the proliferative non-endocrine cells from the culture. Furthermore, they identified integrin α1 as a surface molecule expressed by SC-β. Magnetic cell sorting using an integrin α1 antibody enabled the authors to obtain cultures containing 80% of SC-β.

Endocrine induction — the formation of hormone-producing cell types in the pancreas — is a key step in the differentiation of islet cells from stem cells. The authors' gene-expression analysis of approximately 50,000 single cells allowed them to reconstruct the lineage relationships between human pancreatic progenitor cells and differentiated endocrine-cell types using a previously developed computational approach called pseudotemporal ordering[7] (Fig. 1). It also revealed the order in which these cell types emerge, and the dynamic molecular changes that occur along each differentiation trajectory. Other studies have used scRNA-seq and computational analysis to produce time series (rather than single temporal snapshots) and to model the population dynamics of cells involved in embryonic developmental processes[8,9]. This combined approach could be used to optimize the timing of the various steps in differentiation protocols.

How close are we to a therapeutic product for β-cell replacement? A clinical trial is already ongoing to test the safety and efficacy of transplanting stem-cell-derived pancreatic progenitor cells into people with type 1 diabetes, to restore control of glucose levels in the blood (see go.nature.com/2feyyud). However, transplanted pancreatic progenitor cells still need to differentiate and mature into β-cells to achieve effective glucose-stimulated insulin secretion. Given that we cannot control the signalling factors to which the cells are exposed *in vivo*, it is desirable to develop a product that secretes insulin in response to glucose when the cells are still in culture, and that becomes effective as soon as they are transplanted[5]. Moreover, transplanting only SC-β might not be sufficient to treat type 1 diabetes, because α-cells are also essential for tight control of hormone secretion from human islets, and for overall regulation of glucose levels.

Veres *et al.* have made great progress in addressing several of these problems. They provide a detailed scRNA-seq data set that covers the major steps of *in vitro* differentiation of islet cells, which will be useful for characterizing the cells produced when signalling factors are changed in different protocols. One key finding of their study is that SC-α and SC-β can be generated together, but that gut endocrine SC-EC and proliferative non-endocrine cells also emerge. This finding will inform the production of a defined and safe therapeutic product, which will require SC-α and SC-β to be isolated with a high level of purity, and proliferative progenitor cells removed to avoid the risk of cancer. Finally, the authors' detailed analysis of the scRNA-seq data has led to the identification of novel surface markers and signalling pathways that will further improve islet-cell differentiation and purification procedures. Overall, this study gets us a step closer to β-cell replacement in the clinic. ■

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CANCER

Manipulating a tumour suppressor

Tumours often become resistant to treatment, but how this occurs is poorly understood. An analysis of how the protein Rb affects tumour growth and the response to therapy might cast light on the problem. SEE LETTER P.424

SETH M. RUBIN & JULIEN SAGE

The development of cancer invariably involves cellular proteins that have unwanted effects or that become inactive. Therapeutically targeting proteins that are more active than normal is conceptually straightforward — a drug needs to be found that, like a wrench thrown into a machine, halts the malicious activity. By contrast, remedying the loss of a type of protein called a tumour suppressor, which normally opposes cancer growth, presents a conundrum. How can a protein be targeted by drugs if it is no longer present? One way of getting around this problem and finding new drug targets is to identify other effects, such as molecular changes, that occur when a tumour suppressor is lost or does not function. On page 424, Walter *et al.*[1] report their analysis of the role of the tumour-suppressor protein retinoblastoma (Rb), using an experimental system that allowed them to engineer the loss and reactivation of this protein in lung cancer in mice.

Since the discovery of Rb's tumour-suppressor activity from studies of children with a type of eye cancer, and the subsequent identification of Rb loss or inactivation as a characteristic of many human tumours, this protein has served as a model for studying tumour suppressors in cancer[2]. Rb can act as a key brake on cell-cycle progression, and it promotes cellular differentiation into specific cell types, which slows the development of cancer. Many cancers block Rb function by increasing the activity of enzymes called cyclin-dependent kinases (Cdks), which inactivate Rb through phosphorylation[3] (the addition of phosphate groups to it; Fig. 1a). Anticancer treatments involving inhibitor drugs that target Cdk4 and Cdk6 to prevent them from inactivating Rb have shown some

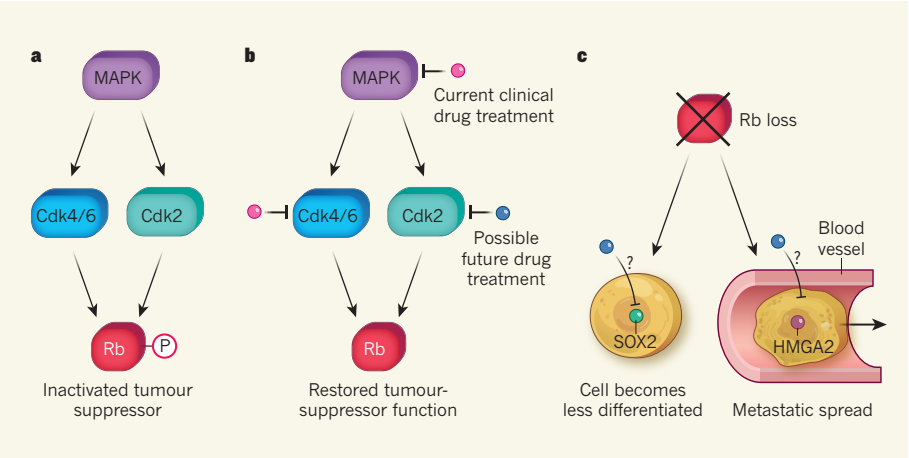

Figure 1 | The Rb protein in cancer cells. **a**, Rb is a type of protein known as a tumour suppressor, which can help to block cancer development. A common feature of lung cancer is Rb inactivation by the addition of phosphate groups (P) to the protein. This frequently occurs through a pathway that requires various enzymes: MAPK; Cdk4 and Cdk6; or Cdk2. **b**, Drugs that block the action of MAPK or Cdk4 and Cdk6 are in use in the clinic or under development for lung cancer treatment. Walter *et al.*[1] studied a mouse model of lung cancer, and report that Cdk2 has a major role in inactivating Rb. Therefore, the development of drugs that target this protein might have promise in treating lung cancer. **c**, Rb expression is often lost in lung cancer cells (yellow). The authors studied the effect of Rb loss in mouse models of the cancer. The absence of Rb resulted in loss of cellular differentiation and an increase in the spread of cancer cells to other sites in the body through a process called metastasis. Proteins implicated in such changes, such as SOX2 and HMGA2, might offer targets for drug development.

success. However, as would be expected, these inhibitors are not effective if Rb is not expressed in the tumours[4].

Walter and colleagues studied the effects of Rb loss in a mouse model of a common form of lung cancer called lung adenocarcinoma. The authors used genetically engineered animals to control whether and when Rb was made in lung cancer cells. This showed that the absence of Rb (a state termed an Rb knockout) promotes cancer development by causing an increase in both tumour growth and its spread to secondary sites in a process called metastasis. The results provide some of the strongest *in vivo* evidence so far to support a role for Rb loss in driving not only cancer initiation, but also its progression.

The authors observed that the molecular changes that lead to progression and metastasis in this lung cancer model are strikingly different, depending on whether Rb is present or absent. If Rb was missing, compared with the situation in which Rb was present but inactivated by Cdks, the cancer cells had an increased propensity to change their cellular identity to a less-differentiated state that allowed the disease to progress more rapidly. This state was accompanied by the expression of metastasis-promoting proteins such as HMGA2. Such cellular dedifferentiation in the absence of Rb has also been observed in prostate cancer[5]. Although the precise mechanism that controls cellular plasticity in this scenario is unknown, it might involve the activation of factors involved in cellular reprogramming, such as the transcription-factor protein SOX2, which is normally inhibited by Rb (ref. 6).

Building on previous studies[7,8] that investigated Rb loss in mouse models of cancer, Walter and colleagues studied the effect of reinstating Rb expression in mice with lung cancer months after the Rb-encoding gene had been deleted by genetic engineering. They found that re-expression of Rb promoted the redifferentiation of undifferentiated cancer cells, rendering the tumours less malignant. This observation is important, because it suggests that strategies to reactivate Rb or to manipulate proteins acting downstream of Rb might be therapeutically beneficial for treating cancers that have lost Rb expression.

Another key observation made by Walter and colleagues is that tumours that lack Rb do not upregulate a signalling pathway containing the kinase MAPK, which is often activated in lung adenocarcinoma. This had not been reported previously and is unexpected, given that upregulation of the pathway is a hallmark of this type of cancer. The authors' data strongly suggest that inactivating Rb is a major objective of MAPK activation in lung cancer that is no longer required in the absence of Rb.

The clinical success of Cdk4 and Cdk6 inhibitors indicates that manipulation of Rb activity can stop cancer progression. However, the development of resistance to such therapies is motivating the search for other drugs that could mimic the reactivation of Rb function, and for new targets in combating cancer cells that have lost Rb expression. Walter and colleagues' success in identifying molecular and cellular effects of Rb loss, such as cellular dedifferentiation and an absence of upregulation of MAPK signalling, will help with these efforts.

MAPK signalling can inactivate Rb through several mechanisms[9]. Walter and colleagues' results indicate that Rb inhibition results from increased MAPK signalling that inactivates[10] a Cdk inhibitor protein called p27, thereby activating Cdk2 — a Cdk that can phosphorylate and thus inactivate Rb (ref. 3). The authors' observations regarding the interplay between MAPK and Cdk2 implicate Cdk2 as a potential drug target in lung cancers that retain Rb but are resistant to Cdk4 and Cdk6 inhibitors. In this context, the combined inhibition of MAPK and Cdk2, or of Cdk4, Cdk6 and Cdk2, might potently stop the growth of lung tumours by ensuring that Rb remains in its active state and is not associated with high levels of phosphorylation (Fig. 1b).

However, more work is needed to identify further potential drug targets. Efforts should also be made to determine the sensitivity of a person's cancer to inhibitors of Cdk4 and Cdk6 or Cdk2, including assessing the individual's genetic profile and monitoring alterations termed epigenetic changes — modifications to DNA or DNA-binding proteins that can affect gene expression. Furthermore, it would be useful to identify targetable proteins or pathways that inhibit the cellular dedifferentiation that occurs after Rb loss (Fig. 1c).

Finally, it will be essential to understand to what extent the principles of lung cancer development and progression are applicable to other types of cancer. The experimental system used by Walter *et al.* could help to address this challenge, because their results might elucidate the effect of loss of tumour-suppressor proteins in diverse contexts. Studies of Rb have long provided a useful model system in which to investigate fundamental mechanisms of tumour suppression. Now they might also offer a way forward in uncovering new anticancer strategies. ■

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