Title

Manipulating the tumour-suppressor protein Rb in lung cancer reveals possible drug targets

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Authors

Rubin, Seth M

Sage, Julien

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Tumours often become resistant to treatment but how this occurs is poorly understood. An analysis of how the protein Rb affects tumour growth and therapy response has implications for cancer treatment and drug development. See Letter p.XXX

Seth M. Rubin & Julien Sage

The development of cancer is invariably accompanied by cellular proteins performing unwanted activities or losing their function. 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, specifically 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 to get around this problem and find new drug targets is to determine other consequences, such as molecular changes, that occur when a tumour suppressor is lost or does not function in a particular type of cancer. Writing in *Nature*, Walter *et al*.[1] report their analysis of the role of the tumour suppressor

protein retinoblastoma (Rb) using both an experimental system that allowed them to engineer the loss and re-activation of this protein in lung cancer in mice and human cancer cells grown *in vitro****[OK?]***.

Since the discovery of the tumour-suppressor activity of Rb 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 that halts cell-cycle progression and it promotes cellular differentiation into specific cell types. Many cancers block Rb function by increasing the activity of enzymes called cyclin-dependent kinases (Cdks), which add phosphate groups to Rb to inactivate it[3]. There has been some success in the clinic with anticancer treatments that use inhibitor drugs that target Cdk4 and Cdk6 and prevent them from inactivating Rb. However, as would be expected, these inhibitors are not effective if Rb is not expressed, which might happen as a tumour evolves***[OK? (or please revise to provide your preferred comment about Rb loss in tumours (for example, is Rb loss a common occurrence when treatment resistance occurs?)***][4]. A clear understanding of the consequences of Rb inactivation is lacking, which limits our ability to target tumours in which Rb is absent.

Walter and colleagues studied the consequences of Rb loss in a mouse model of a common form of lung cancer called lung adenocarcinoma. The authors genetically engineered the animals so they could control whether and when Rb was made in lung-cancer cells. This allowed them to make a key observation that the absence of Rb (a state termed an Rb knockout) promotes cancer development by causing an increase in

both tumour growth and tumour spread to secondary sites in a process called metastasis. This provides some of the strongest *in vivo* evidence provided so far supporting a role for Rb loss not only in driving cancer initiation but also in promoting cancer progression.

The authors observed that the molecular changes that lead to cancer progression and metastasis in this lung cancer model are strikingly different depending on whether Rb was present or absent. If Rb was missing, compared to the situation when Rb was present but inactivated by Cdks, the cancer cells had an increased propensity to change their cellular identity to a less-differentiated state, which allows the disease to progress more rapidly. Cellular dedifferentiation in the absence of Rb has also been observed in prostate cancer[5]. Although the precise mechanism controlling 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[6].

Building on previous studies[7,8] that investigated the consequences of Rb loss in mouse models of cancer, Walter and colleagues have deepened our understanding of Rb function by studying the effect of reinstating Rb expression in lung cancer months after the Rb-encoding gene was deleted by genetic engineering. This re-expression of Rb promoted the re-differentiation of undifferentiated cancer cells, thereby rendering the tumours less malignant. This observation is important because it suggests that strategies to re-activate Rb or to manipulate proteins that act downstream of Rb might have therapeutic benefits for the treatment of cancers that have lost Rb expression.

Another key observation made by Walter and colleagues is that tumours that lack Rb surprisingly do not upregulate the signalling pathway that contains the enzyme MAPK kinase, which is a pathway that is often activated in lung adenocarcinoma. This observation regarding the MAPK pathway had not been previously reported and is unexpected given that the upregulation of this pathway is a hallmark of this type of cancer. Their data strongly suggest that inactivation of Rb function is a major role of MAPK activation in lung cancer. MAPK signalling can cause Rb inactivation by several different mechanisms[9]. Walter *et al*. indicate that an increase of MAPK kinase signalling above normal in lung adenocarcinoma causes Rb inhibition because inactivation[10] of the Cdk inhibitor protein called p27 causes the activation of Cdk2 — a Cdk family member that can[3] phosphorylate and inactivate Rb.

The success of inhibitors of Cdk4 and Cdk6 in the treatment of some tumours indicates that the manipulation of Rb activity can stop cancer progression. However, the observation of therapy resistance is motivating the search for other drugs that could re-activate Rb and for the identification of additional targets that could be used in treatments for cancer cells that have lost Rb expression. Walter and colleagues' success in identifying molecular and cellular consequences of Rb loss, such as cellular de-differentiation and an absence of up-regulation of MAPK signalling, will help with these efforts.

The authors' observations regarding the interplay between MAPK and Cdk2 implicates Cdk2 as a potential drug target in lung cancers that have Rb but are

nevertheless resistant to inhibitors of Cdk4 and Cdk6. In this context the combined inhibition of MAPK and Cdk2, or the combined inhibition of Cdk4, Cdk6 and Cdk2 might potently stop the growth of lung tumours by ensuring that Rb is not deactivated by phosphorylation (Fig. 1). However, more work will be needed to identify additional 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 a person's genetic profile and monitoring alterations termed epigenetic changes that are modifications of DNA or DNA-binding proteins. It would be useful to try to identify targetable proteins or pathways that inhibit the cellular dedifferentiation that occurs upon Rb loss.

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 be relevant for understanding the effect of loss of tumour suppressor proteins in diverse contexts***[OK?]***. Studies of Rb have long provided a useful model system to investigate fundamental mechanisms of tumour suppression. Now they might also offer a way forward to uncover new anti-cancer strategies.

Seth M. Rubin *is in the Department of Chemistry and Biochemistry, University of California, Santa Cruz, Santa Cruz, California 95064, USA*. **Julien Sage** *is in the Departments of Pediatrics and of Genetics, Stanford University, Stanford, California 94305, USA*.

e-mails: srubin@ucsc.edu; julsage@stanford.edu

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Figure 1 | Rb protein in cancer cells. a, Rb is a type of protein known as tumour suppressor that can help to block cancer when it is active. A common feature of lung cancer is Rb inactivation by the addition of phosphate groups (P) to the protein. This can occur through a pathway that requires the action of the enzyme MAPK and the enzymes Cdk4 and Cdk6 or the enzyme Cdk2. **b**, Drugs currently in use in the clinic for lung cancer (green) block the action of MAPK or Cdk4 and Cdk6. Walter *et al*.[1] studied a mouse model of lung cancer and report that Cdk2 has a major role in the inactivation of Rb. Therefore the development of drugs that target this protein might have promise as a new way to treat lung cancer. **c**, Rb expression is often lost in lung cancer. Walter and

colleagues studied the consequence of Rb loss that they engineered in mouse models of lung cancer. This resulted in a 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, like the proteins Sox2 or HMGA2, might offer targets to be investigated for future drug development.