IMMUNOLOGY

Deregulation required?

Patients with cancer have detectable numbers of T cells in their blood that recognize tumour-associated antigens, yet this rarely corresponds with effective tumour eradication, even after tumour-vaccine-induced increases in cytotoxic T-cell numbers. Curiel and colleagues now show that regulatory T cells might be one factor that prevents an effective antitumour response.

Recent studies have indicated that when self-recognizing T cells are present, regulatory T cells become activated and shut down the antitumour response. Curiel and colleagues studied the profile of tumour-associated T cells in patients with advanced ovarian cancer and found that 10–17% of T cells isolated from the tumour ascites were regulatory T cells ($CD4^+CD25^+$). Furthermore, about 23% of the T cells identified in the tumour tissue itself were of the regulatory subtype, with the percentage of these cells increasing as disease progressed. However, regulatory T cells were undetectable in normal ovarian tissue sections.

But what effect do regulatory T cells have on the antitumour immune response? The authors showed that regulatory T cells isolated from human tumour ascites inhibited the proliferation of autologous tumour-specific cytotoxic T cells in a mouse xenograft model of ovarian cancer. And although injection of tumour-specific cytotoxic T cells delayed tumour growth, this effect was inhibited by the co-injection of regulatory T cells.

What might attract a regulatory T cell into the tumour tissue? Regulatory T cells are normally localized in lymph nodes, but surprisingly, there were fewer regulatory T cells in tumour-draining nodes than in lymph nodes from patients without cancer. Indeed, with increasing cancer progression, the levels of regulatory T cells decreased in the lymph nodes and increased within the tumour tissue and ascites, indicating that regulatory T cells might be actively recruited from these sites by

TUMORIGENESIS

Two faces of hypoxia

Hypoxia is often thought of as something cancer cells need to overcome to survive, necessitating the formation of new blood vessels to escape from areas of low oxygen. However, a growing body of evidence indicates that hypoxia might actually promote cancer development. Adding to this evidence, a recent study from Eileen White and colleagues shows that hypoxia drives cancer progression by promoting genomic instability, and that inactivation of apoptosis is essential for tumour-cell survival during this process.

The authors were interested in the role of the pro-apoptotic BCL2-family proteins BAK and BAX in tumorigenesis. In baby mouse kidney epithelial cells transformed with the adenoviral *E1A* oncogene and a dominant-negative form of *TP53*, apoptosis is partially blocked — because of p53 inactivation — but the p53-independent apoptotic pathway that involves BAX and BAK is still functional. These cells are transformed *in vitro*, but do not form tumours when injected into animals, raising the question of why inactivation of the BAX/BAK apoptotic pathway is required for tumorigenesis.

White and colleagues investigated whether this is because these cells that express functional BAK and BAX are unable to survive in the hypoxic conditions that tumour cells encounter *in vivo*. Consistent with this, they found that the injected cells were subjected to high levels of hypoxia, resulting in cell death by apoptosis and necrosis. By contrast, despite being exposed to hypoxic conditions *in vivo*, cells in which the BAX/BAK pathway had been inactivated — either by deletion of *Bax* and *Bak*, or by overexpression of the anti-apoptotic protein BCL2 — survived to form tumours.

Interestingly, cells lacking BAX and BAK function formed giant polyploid tumour cells *in vivo*, whereas cells with functional BAX and BAK did not. This led White and colleagues to suggest that hypoxia promotes polyploidy, but that blocking the p53-independent apoptotic pathway is required for cells to survive this process.

To directly test whether inactivation of the BAX/BAK pathway is required for survival in hypoxic conditions, the authors used an *in vitro* ischaemia assay, in which cells are deprived of both oxygen and nutrients. Transformed cells expressing E1A and dominant-negative p53, but with functional BAK and BAX, underwent apoptosis in these conditions, whereas cells in which the BAK/BAX pathway was inactivated survived.

To test if this promoted genomic instability, the authors subjected cells to ischaemia, followed by a period of recovery, and looked at chromosome content and levels of apoptosis. Ischaemic conditions resulted in the generation of cells with increased chromosome content, regardless of the status of the BAX/BAK pathway. However, for cells in which this pathway was active, high levels of apoptosis were seen, and no polyploid cells were observed after the recovery period. By contrast, inactivation of BAX and BAK function led to the survival and persistance of polyploid cells.

Polyploidy is thought to be an important step in generating the genomic instability and aneuploidy that have been linked to tumour progression. So, it seems that by promoting this process, hypoxic and ischaemic conditions have a key role in cancer progression, but that loss or blockcade of the BAX and BAK pathway is required for survival during this step. This provides a new insight into the molecular changes that are needed for tumours to evolve, and might have important implications for therapeutic strategies that aim to kill cancer cells by inducing hypoxia.

Louisa Flintoft

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WEB SITE
Eileen White's lab:
http://white.cabm.rutgers.edu/index.html

tumour-produced chemotactic factors. The authors examined a number of candidate chemokines and showed that CCL22, produced by both macrophages and tumour cells, attracts regulatory T cells, which express the CCL22 receptor CCR4. An antibody against CCL22 prevented trafficking of regulatory T cells to human ovarian tumours in the xenograft model.

Finally, Curiel and colleagues correlated regulatory T-cell numbers with patient prognosis — a review of 70 patients with ovarian cancer confirmed that the greater the number of tumour-associated regulatory T-cells, the more adverse the prognosis for all tumour stages. The authors conclude that inhibiting the function of regulatory T cells, or perhaps blocking tumour chemokine signalling to inhibit their migration, could improve the efficacy of many antitumour vaccines.

Nicola McCarthy

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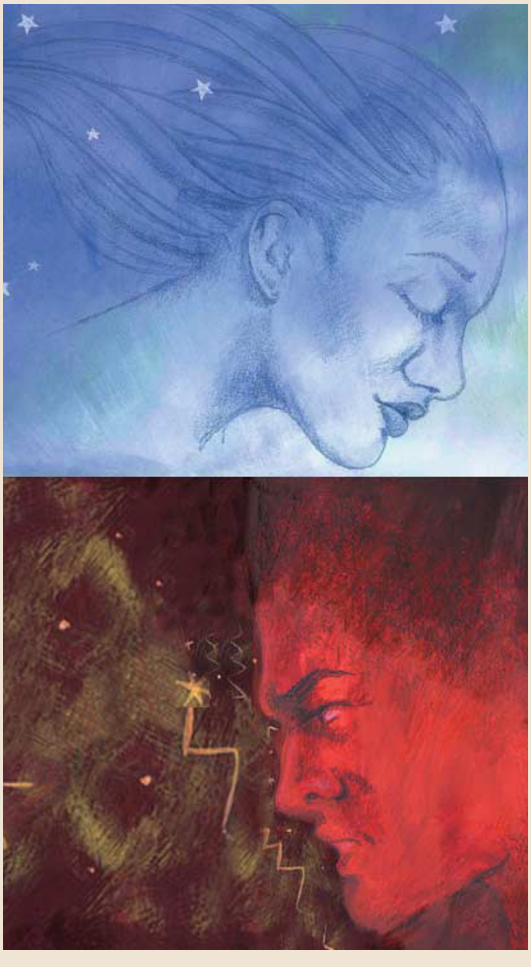

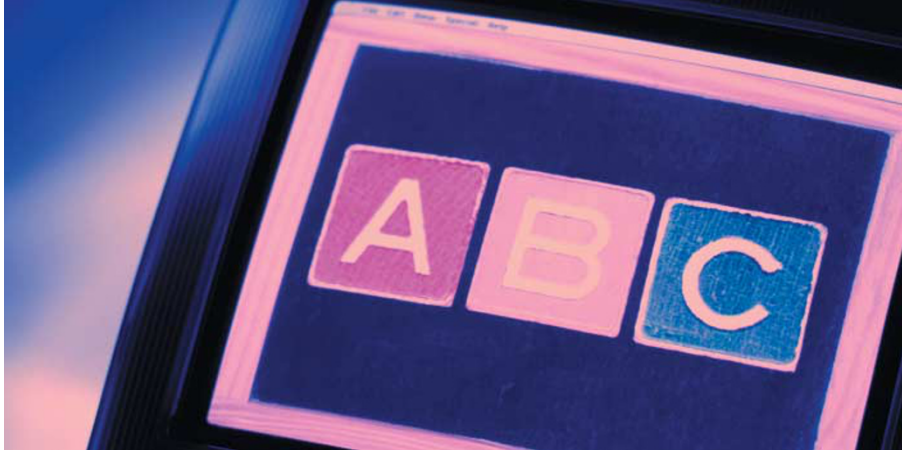

PHARMACOGENOMICS

ABCs of drug resistance

Multidrug resistance resulting from the activity of members of the ABC (ATP-binding cassette) family of membrane transporters, which actively pump many types of anticancer drugs out of cancer cells, is a significant barrier to effective cancer chemotherapy. However, efforts to overcome this problem have been hampered by limited knowledge of many members of the ABC transporter family. Now, as reported in *Cancer Cell*, Szakács *et al.* have coupled expression data for all human ABC transporters in cancer cells with information on the sensitivity of the cells to various anticancer drugs, providing a valuable tool for improving understanding of drug resistance mediated by these proteins.

Of the 48 human ABC transporters, only ~10 have been associated with cancer-drug resistance, such as the archetypal multidrug transporter ABCB1 (also known as MDR1 and P-glycoprotein). However, the high degree of sequence similarity between these ABC transporters indicated that further members might also export cancer drugs. So, Szakács *et al.* set out to explore this possibility by characterizing the expression of ABC transporters in a set of cancer cells whose responses to a large number of compounds are well-characterized: the panel of 60 cancer cell lines at the National Cancer Institute, which have been used to screen >100,000 compounds.

By using real-time PCR assays, which are more specific and sensitive than microarrays, to quantify the levels of messenger RNA of all 48 human ABC transporters, the authors obtained precise correlations between ABC-transporter expression and known patterns of drug activity for 1,429 compounds across the 60 cancer cell lines. As expected, good agreement was found between expression of ABCB1 and reduced cellular sensitivity to anticancer drugs that are known to be substrates for this transporter. Furthermore, using this method, several compounds that were not previously known to be substrates of ABCB1 were identified as potential substrates of this transporter, and these predictions were validated with follow-up experiments. And unexpectedly, some compounds were predicted — and subsequently confirmed — to have their anticancer activity potentiated by ABCB1, rather than antagonized; these compounds might serve as leads for drug development.

In total, the data revealed 131 drug–transporter pairs in which transporter expression correlated with resistance to the drug. As well as transporters known to be associated with drug resistance, several transporters that were not previously implicated in drug resistance were identified; again, follow-up experiments confirmed some of these predictions.

So, overall, the database created by the authors provides a resource for identifying transporters whose expression confers drug resistance, and compounds whose effects are antagonized, unaffected or even potentiated by transporter expression, which will be useful in developing strategies to address the problem of multidrug resistance. The database will also be valuable for future data mining to aid in studies of the function of the many ABC transporters that are not well characterized.

Peter Kirkpatrick

Senior Editor, Nature Reviews Drug Discovery

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