CARCINOGENESIS

Catch-22

BCL2 overexpression leads to inhibition of apoptosis, so promoting carcinogenesis. Now, Ho Jin You and colleagues show that BCL2 also stimulates mutagenesis through its independent function in cell-cycle arrest, by suppressing DNA repair.

The authors first investigated the effect of treatment with the alkylating agent MNNG on a cell line with inducible BCL2. When BCL2 expression was induced the cells were more resistant to MNNG-induced death than control cells, and spontaneous mutagenesis and mutagenesis caused by MNNG were also higher. Decrease of mismatch-repair (MMR) activity correlated with the degree of induction of BCL2. Cells expressing a BCL2 mutant that is unable to induce cell-cycle arrest were more resistant to MNNG mutation than either control cells or cells expressing an anti-apoptotic deficient BCL2 mutant. Moreover, only cells expressing the anti-apoptotic deficient BCL2 mutant had reduced MMR activity. This indicates that BCL2-induced cell-cycle arrest is important for the BCL2-mediated suppression of MMR, but that the anti-apoptotic function is not involved.

So how does BCL2 suppress MMR? The authors discovered that the mRNA of the MMR protein MSH2 was significantly decreased in cells after BCL2 induction. As *MSH2* is an E2F-responsive gene, the authors investigated whether inactivation of this transcription factor could mediate the BCL2-induced suppression of MSH2. BCL2-expressing cells had more E2F1 bound to phosphorylated RB — which keeps E2F1 inactive — than when BCL2 expression was not induced, and binding of E2F1 to the *MSH2* promoter was reduced. Moreover, when small interfering RNAs that target E2F1 were added to the cells, MSH2 espression and MMR activity were reduced in BCL2-expressing cells.

You and colleagues confirmed that the same mechanisms of MMR suppression occurred in two human B-cell lymphoma cell lines that overexpress BCL2, but not in another B-cell lymphoma cell line that does not express BCL2. These data indicate that far from cell-cycle arrest protecting cells from oncogenesis, it actually induces mutagenesis. These findings might help to explain why cancer incidence increases exponentially with the ageing process, in which senescent cells (with irreversible growth arrest) accumulate.

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References and links

ORIGINAL RESEARCH PAPER You, C. *et al*. Bcl-2 expression suppresses mismatch repair activity through inhibition of E2F transcriptional activity. *Nature Cell Biol.* 26 Dec 2004 (doi:10.1038/ncb1215)

WEB SITE

Ho Jin You's lab: http://www.chosun.ac.kr/~rcpm/korea/si_pe(3).htm

TRIAL WATCH

Metastasis profiles

A gene-expression signature of primary tumours from patients with head and neck squamous-cell carcinoma (HNSCC) is better at detecting the presence of lymph-node metastases than the current diagnostic procedure. This study is the first to independently validate a primary tumour expression signature that can reliably detect the presence of metastases in local lymph nodes, and might offer patients better therapeutic options.

To identify a predictive gene-expression signature for lymph-node metastases, Frank Holstege and colleagues analysed 82 primary HNSCC tumours, 45 from patients whose tumours were known to be metastatic and 37 from patients known to be lymph-node negative (N0). Genes expressed differentially in at least 30 samples were selected (1,986 genes) and used to build a predictor. A supervised classification approach was applied to establish a classifier without bias towards the training set, and 102 genes were found to be optimal in predicting the presence or absence of lymph-node metastases.

The accuracy of this set of genes was validated on independent tumours. Expression profiles were generated for an additional 22 samples, and the metastatic status of 19 of these was accurately predicted, with no false negatives. This predictor performed better than the current clinical procedure of lymph-node biopsy in these patients (86% versus 68%). The authors also observed that performance increased when more recent samples were analysed, indicating that long-term storage of tumour tissue has adverse effects on gene-expression profile analysis.

Detecting local lymph-node metastases is important in patients with HNSCC, as their presence or absence determines the treatment regimen. Most patients have the primary tumour removed and patients with lymph-node metastases undergo the additional surgical removal of lymph nodes in the neck and other associated muscles, veins and nerves in the region — termed 'radical neck dissection'. Only 10–20% of patients are considered to be N0, although this status is difficult to determine — one-third of patients diagnosed as N0 are found to not be so. As a result, most clinics perform a selective neck dissection on these patients, which involves removal of a restricted set of lymph nodes. Although less rigorous than radical neck dissection, this procedure can cause many complications and results in overtreatment for patients who are truly N0 and undertreatment for patients who are later found to have lymph-node metastases. The findings of Holstege and colleagues can therefore save many patients from unnecessary surgery and significantly improve the treatment for patients who are currently incorrectly diagnosed as N0.

The experiments also identified possible new metastasis-associated genes. Over half of the predictor genes have not been previously associated with tumorigenesis or metastasis, providing starting points for new investigations. These included genes encoding extracellular-matrix and cell-adhesion components, such as members of the plakin family. Surprisingly, more genes in the predictor set were downregulated than upregulated, indicating that a loss of many cellular activities is also an important aspect of metastasis.

ORIGINAL RESEARCH PAPER Roepman, P. *et al*. An expression profile for diagnosis of lymph node metastases from primary head and neck squamous cell carcinomas. *Nature Genet.* **37**, 182–186 (2005)