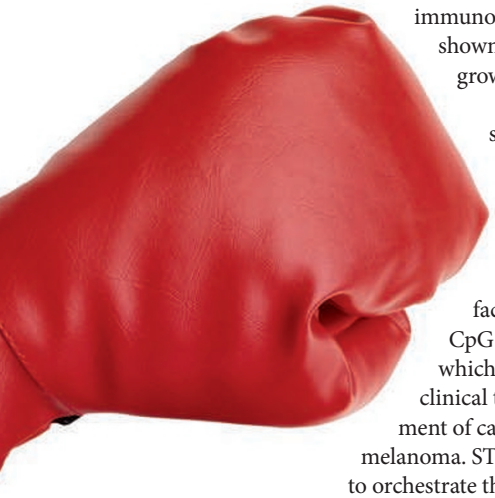

CANCER

CpG–siRNA deals double blow to tumours

Tumours can avert an immune response and boost their own growth by inducing the expression of immunosuppressive, angiogenic and growth factors in neighbouring cells. Reporting in *Nature Biotechnology*, Kortylewski and colleagues now present a new strategy to alter the balance in the tumour microenvironment to re-awaken an anticancer immune response. By coupling a Toll-like receptor (TLR) agonist to small interfering RNA (siRNA), the double blow of TLR-mediated immune activation and targeted silencing of immunosuppressive genes was shown to inhibit tumour growth in mouse models.

The authors coupled siRNA that targets signal transducer and activator of transcription 3 (STAT3), an oncogenic transcription factor, to TLR9-specific CpG oligonucleotides, which are currently in clinical trials for the treatment of cancer, including melanoma. STAT3 has been shown to orchestrate the expression of immunosuppressive and angiogenic factors, contributing to a tumour environment characterized by a lack of tumour-specific cytotoxic T cells, an inhibition of T helper 1 (T_H1) cells and an excess of regulatory T cells, as well as infiltration by myeloid-derived suppressor cells. STAT3 is also persistently activated in many tumour cells of diverse origin, which enhances tumour growth and survival and confers resistance to anticancer therapies. Furthermore, STAT3 is known to attenuate TLR-mediated T_H1-type antitumour responses.

In several mouse models of cancer, peritumoural injection of the CpG–siRNA construct resulted in internalization of the construct by tumour-associated myeloid cells, as well as dendritic cells, macrophages and B cells in tumour-draining lymph nodes. Compared with CpG alone or CpG conjugated to random-sequence siRNA, the CpG–siRNA construct inhibited the growth of subcutaneous B16 and K1735 melanoma cells and CT26 colon carcinoma cells to a greater extent, and induced tumour regression in three out of four mice in the MC38 colon carcinoma model.

In a B16 lung cancer metastasis model, systemic delivery of small amounts of the CpG–siRNA construct (< 1 mg per kg) led to a significant reduction in the number of lung cancer metastases compared with controls. The effects were shown to be immune mediated, as they were reduced in mice depleted of $CD4^+$ and $CD8^+$ T cells and abolished in mice depleted of natural killer cells. Furthermore, analysis of the tumour microenvironment revealed an upregulation of chemokine and co-stimulatory molecule expression by dendritic cells in the tumour-draining lymph nodes, an increase of T_H1-type cytokines, a reduction in regulatory T cell numbers and an increased infiltration of cytotoxic T cells. B16 tumour tissue sections from mice that were treated with CpG–siRNA showed extensive tumour cell apoptosis, which was associated with an increase in tumour-infiltrating neutrophils.

Although there remains scope to optimize the CpG–siRNA constructs, this study shows an elegant way to simultaneously target tumour-associated immune cells to silence tumour-promoting or immunosuppressive molecules, and TLRs to stimulate immune activation. Furthermore, the approach could be broadened by targeting other nucleic acid-binding TLRs, combined with the silencing of different genes, to manipulate the immune response in different cancers and infectious diseases.

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ORIGINAL RESEARCH PAPER Kortylewski, M. *et al. In vivo* delivery of siRNA to immune cells by conjugation to a TLR9 agonist enhances antitumour immune responses. *Nature Biotech.* 13 Sep 2009 (doi:10.1038/nbt.1564)