THERAPEUTICS

Brief encounter

Both preclinical and clinical trials show that anticancer therapies that disrupt the blood supply of a solid tumour are most effective when combined with either radio- or chemotherapy. Jain and colleagues have now found a possible explanation for this result and, surprisingly, it is not that anti-angiogenic factors eradicate the tumour's vasculature, but that they might transiently improve it.

The vascular network feeding a solid tumour is often a mass of highly disorganized and overly large, leaky vessels, leading to an inefficient blood supply and mostly hypoxic tumour tissue. The treatment of solid tumours, such as glioblastoma multiforme, with radiotherapy is hampered by these hypoxic conditions. Jain and colleagues used a mouse orthotopic model of human glioblastoma to assess why combined anti-angiogenic treatment and radiotherapy gives better results.

Initially, the authors combined an antibody (DC101) that inhibits vascular endothelial growth factor receptor 2 (VEGFR2) with γ-radiation and examined tumour growth. Although both treatments together showed an additive response in delaying tumour growth, scheduling radiation treatment 4–6 days after starting treatment with DC101 gave a synergistic response. However, delaying radiotherapy until 8 days after DC101 treatment resulted in loss of this synergy. Jain and co-workers thought that this might be due to a transient improvement in the vascular network, increasing the levels of tumour oxygenation and making the tumour cells more sensitive to radiation.

Analysis of the tumours using *in vivo* multiphoton microscopy and immunofluoresence indicated that the tumour vasculature did change during the initial stages of treatment with DC101 — the vessels became less tortuous and vessel diameter decreased. These changes correlated with the recruitment of pericytes to the tumour vessels — cells that are associated with stabilized vessels in normal tissues. cDNA microarray data, along with mRNA and protein studies showed that angiopoietin-1

VIRAL CARCINOGENESIS

Reactivation

Patients with rheumatoid arthritis and polymyositis treated with the immunosuppresive drug methotrexate (MTX) develop Epstein–Barr virus (EBV)-positive lymphomas more frequently than healthy individuals or similar patients treated with non-immunosuppressive drugs. Wen-hai Feng, Jeffrey I. Cohen *et al.* now report that MTX might promote EBV-positive lymphomas in these patients by a combination of immunosuppression and reactivation of latent EBV.

Latent infection is associated with cellular transformation of B cells, but the lytic form of infection, which leads to release of virus particles and death of the host cell, might also increase the number of latently EBV-infected B cells and increase the likelihood of malignancy. Using pharmacological doses of MTX, the authors showed that MTX induced lytic infection in an EBV-positive latently infected gastric carcinoma cell line (AGS-EBV-GFP) and in EBV-positive lymphoblastoid cell lines (LCLs). Other drugs used to treat rheumatoid arthritis did not have this effect.

To investigate whether MTX induced lytic EBV gene expression directly Feng and Cohen *et al.* transfected EBV-negative cells with the promoters of the early lytic viral proteins BZLF1 and BRLF1 linked to a reporter gene and treated them with MTX — the expression of the reporter gene more than doubled in both experiments. But is this expression accompanied by replication of lytic EBV DNA? Treatment of AGS-EBV-GFP cells with MTX increased the copies of the lytic EBV genome and this effect was inhibited by addition of the antiviral drug acyclovir. This induction of replication seems to be MTX specific, because gemcitabine, which also induced lytic viral gene expression, did not increase replication of EBV DNA. Furthermore, when drug-free medium was taken from MTX-treated AGS-EBV-GFP cells and used to infect an EBV-positive Burkitt's lymphoma cell line, GFP expression was detected in the resulting infected cells, indicating that MTX had induced release of infectious virions.

So what effect does MTX have on EBV loads in patients with rheumatoid arthritis or polymyositis? The mean EBV load in 29 patients receiving treatment regimens including MTX was 40 EBV copies per 10^6 cellular genomes, compared with 5.1 EBV copies in the 12 patients on regimens that did not include MTX.

The authors conclude that the unique ability of MTX to induce EBV replication at the same time as suppressing the immune system might explain the association with EBV-positive lymphomas in patients who already have an increased risk of these malignancies. Whether MTX treatment of cancer patients has any effect on EBV reactivation or any link with EBV-associated tumours should be investigated.

Ezzie Hutchinson

References and links

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Shannon Kenney's lab: http://cancer.med.unc.edu/research/faculty/displayMember.asp?ID=100

expression was selectively increased after treatment with DC101 and that inhibition of the angiopoietin-1 receptor, TIE2, blocked recruitment of the pericytes. In addition, the authors found that the abnormally thick basement membrane often seen in tumour vessels was temporarily reduced. This was not a function of pericyte recruitment, but one of increased matrix-metalloproteinase activity.

Collectively, these results show that blockade of VEGFR2 transiently 'normalizes' the vascular network in tumours and decreases hypoxia, making radiotherapy more effective. The identification of a normalization window in patients might lead to more successful treatments for glioblastoma multiforme and other solid tumours.

Nicola McCarthy

References and links

ORIGINAL RESEARCH PAPER Winkler, F. *et al.* Kinetics of vascular normalization by VEGFR2 blockade governs brain tumour response to radiation: role of oxygenation, angiopoietin-1 and matrix metalloproteinases. *Cancer Cell* 20 Dec 2004 (doi:10.1016/j.ccr.2004.10.011)

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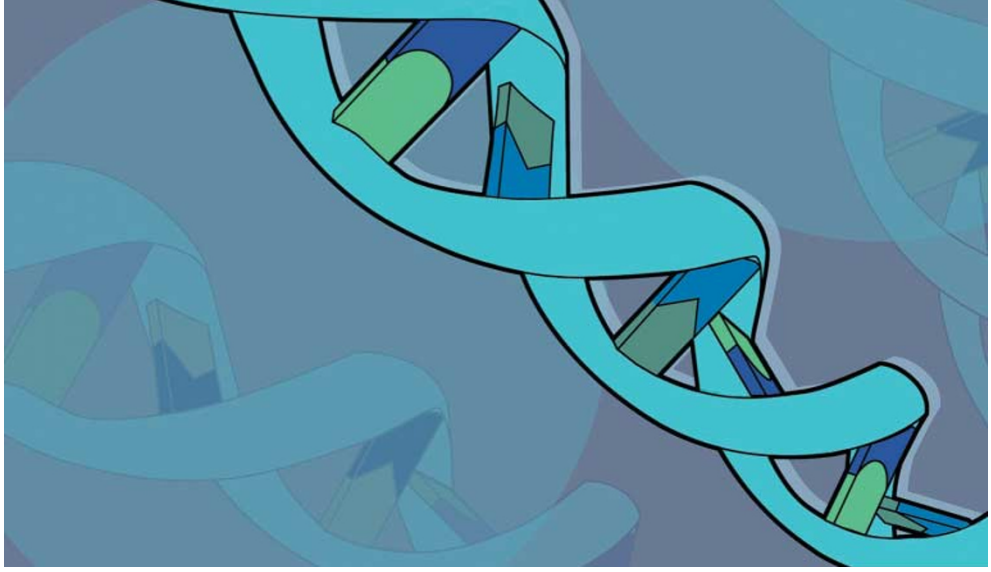

TUMOUR SUPPRESSORS

A new SNP

The *TP53* tumour suppressor is mutated in at least half of all cancers, and other components of the pathway, such as *MDM2* and *ARF*, are mutated in some of the remaining cancers, which emphasizes the importance of the pathway in tumour development. In the latest issue of *Cell*, Arnold Levine and colleagues investigate whether naturally occurring polymorphisms in these components might influence an individual's susceptibility to cancer, and discover that this is, indeed, correct.

The authors focused on a 300 base pair region of the first intron of the intronic promoter of *MDM2* and looked for sequence variation in 50 healthy individuals. They identified a single-nucleotide polymorphism (SNP) — SNP309, a T to G change — that was present at a relatively high frequency. SNP309 lies within an area that contains several putative binding sites for the SP1 transcription factor, and the nucleotide change was thought to extend one of the sites. This might increase the affinity of SP1 binding. The authors analysed this using electromobility shift assays and found that purified human SP1 does bind with greater affinity to oligonucleotides containing SNP309 than to wild-type oligonucleotides. This binding was confirmed *in vivo* using chromatin immunoprecipitation assays.

What is the effect of increased SP1 binding? *Drosophila* SL2 cells, which are deficient in SP proteins, were transfected with SP1 and an expression vector driven by the *MDM2* promoter containing either the wild-type or G/G homozygous variant. The luciferase reporter was expressed at a higher level with the SNP309 variant than with the wild-type promoter. So, it seems that SNP309 does result in higher levels of transcription from the *MDM2* promoter. Tumour-derived cell lines that contain SNP309 were also found to have eightfold higher *MDM2* mRNA expression and fourfold higher protein expression than those with a wild-type *MDM2* promoter. SP1 was shown to be responsible for this increase, as inhibiting its activity with either RNA interference or the antibiotic mithramycin A markedly reduced MDM2 levels.

But how might this SNP affect the p53 pathway? MDM2 is a negative regulator of p53, so its increased expression should attenuate the p53 pathway. This was found to be true, as the p53 response to the chemotherapeutic drug etoposide was different in cell lines that contained SNP309. In cell lines with the wild-type promoter, 20–35% of cells died following etoposide treatment, but in those homozgous for SNP309, only 2–3% of cells died. This low death rate was found to be due to poor induction of the p53 transcriptional programme.

But does this affect the risk of tumour formation? The authors first investigated this in individuals with Li–Fraumeni syndrome — who carry only one germline copy of *TP53*. Those who also possessed SNP309 had higher levels of MDM2 and a weak DNA-damage response. Furthermore, these individuals developed tumours at a younger age and were more likely to develop multiple primary tumours. The SNP309 variant also affects the risk of sporadic cancers, as soft-tissue sarcomas were diagnosed on average 12 years earlier in individuals with the SNP309 variant.

So, naturally occurring genetic variants can influence susceptibility to tumour development. It will be interesting to look for more of these variants in the population.

Emma Greenwood

NPG Executive Editor, Cell Death and Differentiation

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