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Re-educating tumour-associated macrophages

The infiltration of tumours by tumour-associated macrophages (TAMs) is usually associated with an unfavourable prognosis, as they can promote malignancy by suppressing immune responses and stimulating angiogenesis. Reporting in *Cancer Cell*, Rolny and colleagues now show that histidine-rich glycoprotein (HRG) can skew the polarization of TAMs away from their usual pro-tumorigenic (M2) phenotype, towards a pro-inflammatory and anti-angiogenic (M1-like) phenotype, which shrinks tumours and reduces metastasis in animal models.

HRG is an endogenous secreted protein with antitumour activity. This activity has been ascribed to the effects of HRG on tumour vessels, but its exact mechanisms have remained enigmatic. Tumours generally contain lower levels of HRG compared to surrounding tissues, so the authors used a gain-of-function approach to investigate the role of HRG in tumorigenesis.

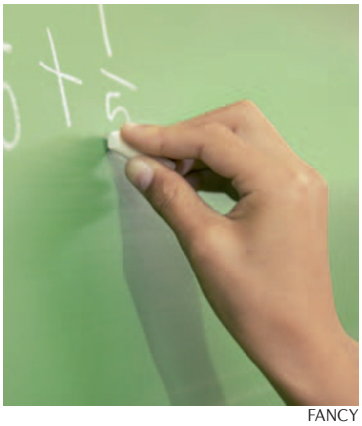
Tumours engineered to overexpress HRG (HRG^+) were shown to grow more slowly and metastasize to a lesser extent in murine hosts. This effect is likely to be mediated via interactions with the tumour environment, as HRG expression had no effect on tumour cell growth *in vitro*.

When examining the effect of HRG on angiogenesis, it was found that vessel density and area were comparable in HRG^+ tumours and control tumours. However, vessels in HRG^+ tumours showed increased perfusion and reduced leakiness, and had a tightened pericyte coat; these parameters are known to be associated with reduced metastasis. Consequently, tumours were less hypoxic, with fewer necrotic and haemorrhagic areas. Antitumour immunity was also improved, with enhanced activity of cytotoxic lymphocytes, natural killer cells and dendritic cells.

Gene expression analysis of TAMs in HRG^+ tumours revealed a skewed polarization towards the anti-tumorigenic and pro-inflammatory M1 phenotype that is often found in regressing tumours. The central role of TAMs for HRG-mediated antitumour responses was illustrated in experiments in which TAMs — indiscriminate of their polarization — were depleted *in vivo* with clodronate liposomes. This treatment decreased the progression and improved the vessel morphology of control tumours, but increased the growth of HRG^+ tumours. This indicates that M2-polarized TAMs are directly involved in generating vessel abnormalities and that TAMs acquire a tumour-suppressive phenotype when they are exposed to HRG.

In vitro experiments confirmed a direct effect of HRG on TAM polarization. This is likely to be enhanced in the tumour environment as hypoxia is known to stimulate the M2 phenotype in TAMs, and improved oxygenation can provide a self-reinforcing stimulus away from the M2 phenotype. The improved oxygenation and perfusion of HRG^+ tumours also led to enhanced tumour killing when the animals were treated with the chemotherapeutic doxorubicin.

When searching for downstream targets, it was found that HRG decreased the expression of placental growth factor (PlGF) in TAMs. The antitumour and antimetastatic effects of HRG were shown to be mediated through PlGF downregulation, as loss of PlGF phenocopied the inhibitory effects of HRG on tumour cells. PlGF also affected TAM polarization, as genetic loss or blockage of PlGF with antibodies, which was comparable to the downregulation of PlGF through HRG, skewed their polarization towards the antitumour M1-like phenotype and led to improved vessel perfusion.



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The authors speculate that HRG recognizes "malignant danger", in line with its presumed role as a pattern recognition molecule, and that tumours respond by downregulating or degrading HRG. Their findings imply that re-educating TAMs by blocking PlGF could be a promising new anticancer strategy. Owing to its effect on tumour perfusion, this could be particularly effective in combination with conventional chemotherapeutic approaches.

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ORIGINAL RESEARCH PAPER Rolny, C. *et al.* HRG inhibits tumor growth and metastasis by inducing macrophage polarization and vessel normalization through downregulation of PlGF. *Cancer Cell* **19**, 31–44 (2011)