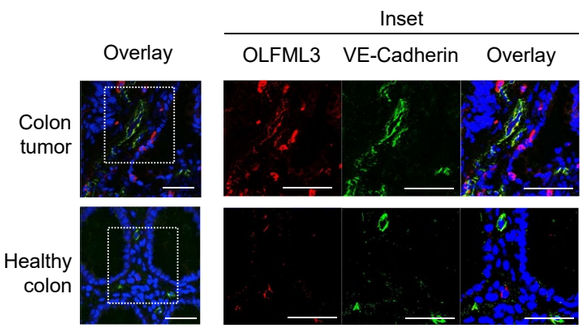
Therapeutically targeting tumour angiogenesis in colorectal cancer

Colorectal cancer is treated by surgery and adjuvant therapy, but some patients relapse. Tumour angiogenesis is a mechanism that promotes disease progression through the formation of new blood and lymphatic vessels. Unfortunately, anti-angiogenesis therapy often isn't successful due to its short-lasting effects and tumour cells' acquired resistance to the treatment. Drs Jimmy Stalin, Curzio Rüegg, Beat A. Imhof and colleagues at the Universities of Fribourg and Geneva, Switzerland, identified two molecules, NOX1 and OLFML3, showing promise as anti-angiogenic combination therapies. The team's research highlights the importance of finding new angiogenic therapeutic targets.

Colorectal cancer (CRC) is a leading cause of cancer-related death. Surgery, often in combination with adjuvant therapy, is the primary treatment of choice. However, many patients will develop recurrent local disease or distant metastasis and will die of the disease. There are various mechanisms by which cancers can progress; in CRC, immune cell recruitment and tumour angiogenesis are the main culprits promoting tumour progression. Tumour angiogenesis is the process of forming new blood vessels that supply the tumour with nutrients and oxygen, which are vital to its further development. Tumours also generate new lymphatic vessels through lymphangiogenesis. Angiogenesis is induced by various molecules, including vascular endothelial growth factor (VEGF), placental growth factor (PGF), and fibroblast growth factor (FGF). In a tumour context, endothelial cells of newly formed vessels are less covered by pericytes compared to vessels in normal tissues, resulting in reduced vascular integrity. This can lead to haemorrhage, immune cells infiltration, and facilitate the ingress of cancer cells into the bloodstream.

Anti-angiogenic therapies are a promising avenue for new cancer therapies. Bevacizumab was the first approved anti-angiogenic drug inhibiting the VEGF family member VEGF-A and is often used in combination with chemotherapy. VEGF-A is involved in the growth and development of tumour-associated blood vessels in many solid tumours, including CRC. However, bevacizumab treatment has a short-lasting and moderate therapeutic effect. This is due to compensatory and resistance mechanisms, including alternative mechanisms of angiogenesis. Interestingly, bevacizumab treatment does not affect pericytes, which surround the blood vessels. This is desirable as pericytes can facilitate blood vessels regrowth into tube-like sleeves left by pericytes after treatment.



Representative immunohistochemistry staining of OLFML3 (red) and VE-cadherin (green) blood vessels in colorectal and healthy tumour from patients.

POTENTIAL ANTI-ANGIOGENIC MOLECULES

Dr Jimmy Stalin and Professor Curzio Rüegg from the University of Fribourg, along with Professor Emeritus Beat A. Imhof at the University of Geneva, Switzerland, have identified the proteins NADPH oxidase 1 (NOX1) and Olfactomedin-like 3 (OLFML3) as novel, potential anti-angiogenic targets for treatment against CRC. The team found them both to be effective targets in combination therapy with immune checkpoint blockade (ICB) therapies, including anti-PD1 therapy.

NOX1 TREATMENT INHIBITS TUMOUR GROWTH

NADPH oxidases (NOXs) are enzyme complexes that support the formation of reactive oxygen species (ROS) enabling normal biological processes, including controlled cell death and angiogenesis. High NOX1 expression has been described to promote tumour growth and metastasis in various cancers. NOX1 is typically expressed at low levels in normal colon cells but gets highly expressed in CRC. NOX1 has previously been found to be implicated in tumour progression through increased inflammation. However, the precise role of NOX1 in cancer is not completely understood.

A NOX1 inhibitor called GKT771, generated by the pharmaceutical company Genkyotex, reduced tumour growth in a murine transplantation model of CRC cancer using the cell line MC38. Treating 200mm^3-sized tumours, representative of established tumours in patients, with GKT771 reduced tumour size by 35% in this model. It suggests that NOX1 inhibition can reduce tumour growth in well-established tumours seen in a clinical setting. This effect was confirmed as MC38 tumours in NOX1-deficient mice also grew more slowly, comparable when wild-type mice were treated with the NOX1 inhibitor. GKT771 hindered tumour angiogenesis in two ways: firstly, by decreasing the number of vessels branching that affected endothelial cell growth and secondly, by reducing expression of the pro-angiogenic factors VEGF-A and PGF. Similar results were seen in NOX1-deficient mice, as the levels of both angiogenic growth factors were lower, and the number of blood vascular cells and endothelial cells were reduced. Taken together, the results show that NOX1 contributes to tumour angiogenesis and tumour growth.

EFFECTS OF NOX1 BLOCKING ON IMMUNE CELL ACTIVITY

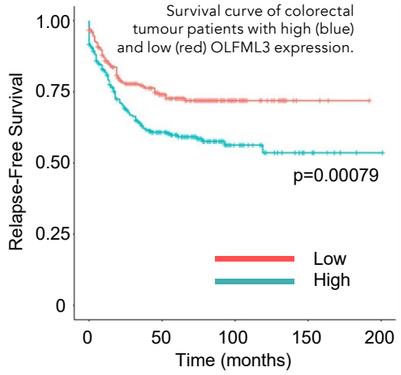
To investigate whether the anti-tumour effects were based on immune cell involvement, researchers looked at the effects of GKT771 on T lymphocytes and natural killer T cells (NKT) in MC38 tumours in mice. As GKT771 did not affect cancer cells themselves in vitro, this suggests that the immune system substantially contributes to the anti-tumour effects of GKT771. GKT771 was used in combination with a blocking anti-IFN-γ antibody that prevents activation of T and NKT cells in the MC38 mouse model. IFN-γ is essential for stimulating anti-tumour immunity of T lymphocytes and NKT cells (white blood cells used in killing tumours). Anti-IFN-γ treatment increased tumour growth and partially reduced the anti-tumour effect of GKT771, suggesting that the GKT771 anti-tumour mechanism may involve IFN-γ and immune cell activation. Further experiments with other reagents were carried out to see if GKT771 anti-tumour effects were based on immune cell involvement.

NOX1 INHIBITION IMPROVES ANTI-PD-1 TREATMENT

Tumours can turn off the immune cells' ability to recognise cancer cells and to recruit lymphocytes to infiltrate the tumour (the so-called tumour-infiltrating lymphocytes, TILs). Anti-PD1 therapy, a type of ICB (immune checkpoint blockade) therapy, relies on the presence of TILs in the tumour to promote cancer-cell killing. However, anti-PD1 therapy is not effective in all tumour cases and often works more efficiently with another treatment.

Researchers found that NOX1 inhibition transforms tumour-associated macrophages into inflammatory types and increases the number of NKT cells in tumours, but does not change the number of TILs. Inflammatory macrophages and NKT cells exert high natural anti-tumour activity and TILs show tumour killing upon activation. Therefore, Stalin and his collaborators investigated the effects of GKT771 on TILs using combinatorial therapy with the T cell activator anti-PD1. They found that GKT771 in combination with anti-PD1 treatment reduced tumour growth more effectively compared to anti-PD1 monotherapy. Two animals out of seven were completely tumour-free at the end of the treatment, due to the cumulating effects of GKT771 including induction of inflammatory macrophages, increased numbers of NKT cells, activation of TILs, and inhibition of tumour angiogenesis.

From these results, the research team concludes that NOX1 inhibition represents a promising and effective anti-tumour strategy in CRC. Still, it is reliant on host NOX1 expression and a functional immune system. Although promising, its anti-angiogenic properties aren't sufficient on their own and need activation of the tumour-associated immune system.



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OLFML3 EXPRESSION IN CRC

OLFML3 is the second potential candidate for anti-angiogenic treatment of CRC that the researchers have investigated. OLFML3 is a protein with pro-angiogenic properties involved in tissue remodelling. OLFML3 has also been implicated in cancers as a key regulator of angiogenesis, immune-cell recruitment, pericyte coverage, and lymphangiogenesis. However, little is known about its therapeutic potential in cancer, including CRC.

An online database was used to obtain expression data of OLFML3 in CRC patients. High OLFML3 gene expression correlated with higher-stage colorectal tumours (stage 2 to stage 4) compared to stage 1. High OLFML3 expression was also correlated with angiogenesis-associated factors. Furthermore, high OLFML3 expression was seen in patient-derived tumours (primary and metastatic) transplanted into mice too, which was also associated with angiogenic factors. CRC patient data revealed that high OLFML3 expression correlated with shorter survival, the CMS4 subtype of CRC with increased angiogenesis and inflammation, and genes associated with pro-angiogenic and

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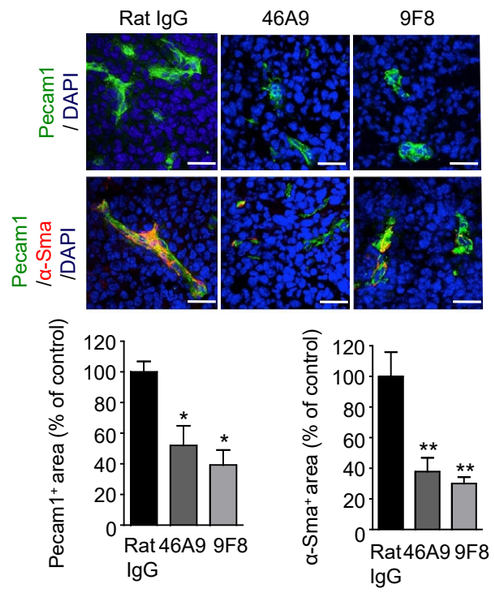

Representative immunohistochemistry staining of Pecam1 (green) endothelial cells and α-Smooth Muscle Actin (red) pericytes forming blood vessels in mouse tumour treated with anti-OLFML3 blocking antibodies (46A9 and 9F8) compared to non-treated tumour (Rat IgG).

lymphangiogenic factors, consistent with a role of OLFML3 in tumour angiogenesis.

OLFML3 INHIBITION DECREASES TUMOUR GROWTH

Various anti-OLFML3 antibodies were used to block OLFML3 activity in mouse MC38 CRC models, resulting in decreased MC38 tumour growth. Pecam1 (expressed on vascular endothelial cells) staining was used to observe tumour blood vessels, and OLFML3 inhibition significantly decreased Pecam1-positive endothelial cells in tumours, demonstrating that anti-OLFML3 antibodies represent potent anti-tumour and anti-angiogenic agents.

OLFML3 INHIBITION REDUCES ANGIOGENESIS

Investigations revealed that angiogenic factor VEGF could regulate OLFML3 in tumours as CRC mouse models treated with bevacizumab (which targets human, tumour-derived VEGF-A) significantly reduced expression of OLFML3, angiogenesis, and lymphangiogenesis-associated genes. This was functionally confirmed by reduced tumour sizes in MC38 cells transplanted into mice and treated with DC101 (an anti-mouse VEGFR-2-blocking antibody preventing the binding of the angiogenic factor VEGF-A). Further analysis revealed that OLFML3 expression in bevacizumab-treated tumour tissue also correlated with the expression of angiogenesis-related genes.

OLFML3 inhibition reduced the expression of angiogenesis associated genes; however, it did not cause a compensatory increase of VEGF-A. OLFML3 treatment also reduced both vascularisation and pericytes coverage in tumours. As pericytes, in addition to tumour-associated endothelial cells, express OLFML3, targeting pericytes is part of the anti-tumour mechanism of OLFML3 inhibition.

Combined anti-PD1 ICB and anti-OLFML3 treatment increased anti-PD1 based therapy efficacy by more than 50%.

OLFML3 INCREASES IMMUNE CELL RECRUITMENT

Researchers looked at the effects of anti-OLFML3 antibodies or mouse OLFML3 genetic deletion on immune cell recruitment in MC38 CRC tumours. A population of macrophages called tumour-associated macrophages (TAMs), which promote tumour growth, were significantly reduced in both OLFML3-deficient mice and mice treated with anti-OLFML3 antibodies. To determine whether a reduction of TAMs is associated with angiogenesis, macrophages were depleted in tumour models using clodronate liposomes – a treatment that induces macrophage death. Treatment with OLFML3 inhibition and clodronate liposomes increased anti-angiogenic effects by reducing angiogenesis and inhibiting tumour growth. In gene expression databases, OLFML3 expression correlated with macrophage-associated factors in human CRC tumours. The factor CSF1, a macrophage proliferation and survival factor, was also correlated with OLFML3 expression, suggesting macrophage involvement in the anti-tumour effects of the OLFML3 inhibition treatment. To test this hypothesis, MC38 CRC tumours were treated with anti-CSF1 blocking antibody. This resulted in reduced tumour growth, suggesting that the anti-tumour effects of OLFML3 may be associated with CSF1-related mechanisms. These findings demonstrate that anti-OLFML3 therapy impairs recruitment of TAMs to CRC tumours and that TAMS are an additional target of anti-OLFML3 treatment.

OLFML3 IMPROVES EFFICACY OF ANTI-PD1 TREATMENT

Researchers found that combined anti-PD1 ICB and anti-OLFML3 anti-angiogenic targeting increased anti-PD1 based therapy efficacy by more than 50%. Three of the eight mice were tumour-free at the end of the experiment. Combination treatment did not affect the recruitment of infiltrating immune cells, but the presence of NK cells was significantly increased in MC38 tumours treated with combination treatment.

Overall, Dr Stalin and his collaborators highlight the therapeutic benefits of antibody-mediated targeting of OLFML3 and small pharmacological molecule inhibition of NOX1, providing essential insight into the anti-tumour mechanisms involving tumour angiogenesis and immune cell infiltration. Both treatments showed promising anti-tumour strategies in CRC, making anti-OLFML3 or -NOX1 attractive therapeutic CRC, especially in combination treatment with anti-PD1 antibodies immune checkpoint inhibitor.

Behind the Research

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Research Objectives

The research of Drs Stalin, Rüegg, and Imhof is focused on discovering angiogenic molecules for therapeutic purposes.

Detail

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Bio

Jimmy Stalin is Maitre-Assistant of experimental pathology at the Department of Oncology, Microbiology, and Immunology at the University of Fribourg, Switzerland. His research includes anti-angiogenic and targeted cancer therapies, including the characterisation of antibodies and chemical compounds for therapy and diagnostic with clinical applications.

Curzio Rüegg trained in medicine and cell biology. Since 2009, he has been Chair of Pathology at the University of Fribourg and before this was Head of Experimental Oncology at the University of Lausanne. His current research focuses on tumour microenvironment, metastasis, and biomarkers in breast cancer. He co-founded two start-ups active in cancer detection.

Beat A. Imhof is a professor emeritus in experimental pathology and was Chair of the Department of Pathology and Immunology at the Medical Faculty, University of Geneva, Switzerland. He investigated inflammatory and angiogenic diseases. He created Abologix, a start-up company developing antibodies for oncological indications.

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Personal Response

Could these molecules potentially be used for treatment in any other types of cancer?

"As tumour angiogenesis is a hallmark of adult solid cancer, NOX1 and OLFML3 could be potential targets in other, angiogenic tumours. Importantly, as tumour angiogenesis is a crucial actor in metastatic diseases, studying how to target these molecules to prevent or treat metastatic disease is of clinical interest. Indeed, most tumour patients died due to metastasis."

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