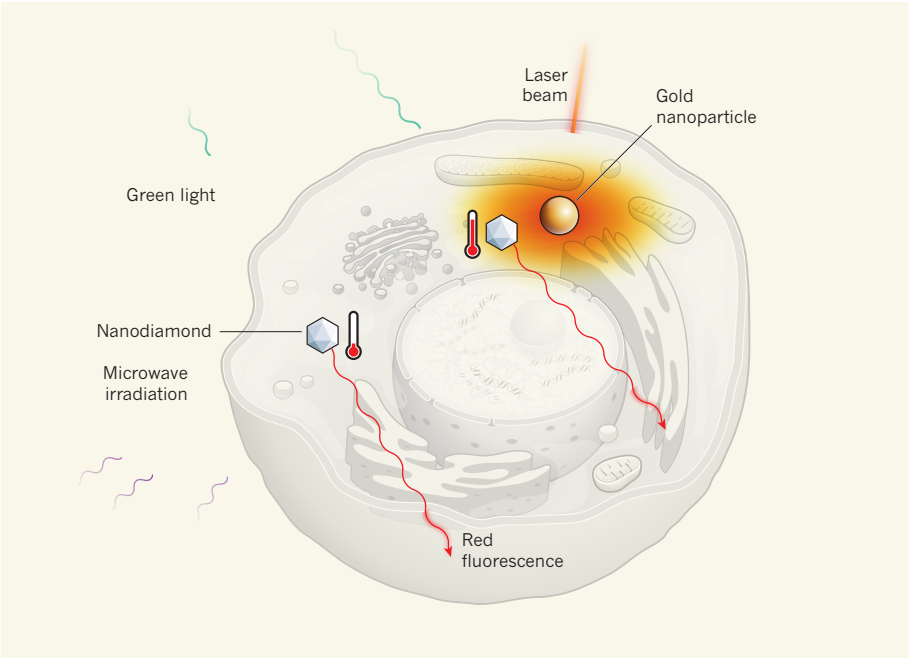

Figure 1 | Temperature measurements in living cells. When diamond-lattice defects known as nitrogen vacancy (NV) centres are excited by green light, they emit red fluorescence. Kucsko *et al.*[1] inserted nanodiamonds into single living cells, and irradiated them with microwaves to modulate the electron occupancy of spin states in NV centres, and with green light. By measuring the fluorescence from these centres, the authors established the electron occupancy of the spin states, and so determined changes in the ground-state energy gap (the microwave frequency that corresponds to the energy difference between spin states) that are associated with temperature variations. In this way, they measured the temperature gradient generated when a gold nanoparticle in the cell was heated by a laser beam, achieving sub-kelvin sensitivity.

best-known thermal conductivity of all solid materials. This is an ideal blend for a nanothermometer. Furthermore, temperature sensing with nanodiamonds could be extended to *in vivo* applications if a different method for fluorescence excitation were adopted: microwave excitation of electronic spin states has already been carried out in animals[5], and the use of 'two-photon' excitation would allow the analysis of deeper tissue than could be achieved with the present method.

Because nanodiamonds are discrete objects, however, the authors' method can take measurements only at distinct locations, rather than taking continuous measurements of a temperature field. Furthermore, the method monitors temperature variations rather than absolute temperature. The authors suggest that this limitation could be overcome by using ensembles of nanodiamonds or diamond samples in which the lattices have low strain, either of which would reduce the experimental variations that currently limit absolute temperature from being measured. At present, the technique also has a fairly low temporal resolution of tens of seconds. This is sufficient for measurements of many biological processes, such as changes in gene expression, but is too slow for studies of temperature effects in faster processes, for example the initial steps of signal transduction, or neural activity.

How might this new tool further our understanding of human cell biology, or enable biomedical advances? Most human cells are 10–20 micrometres in size and are highly compartmentalized by internal membranes that separate cellular organelles. These organelles create multiple micrometre-sized reactors in which a plethora of energy-producing and energy-absorbing reactions occur. The reactions generate intracellular temperature gradients on micrometre and submicrometre scales that, in turn, influence other cellular biochemical reactions. Furthermore, external biochemical signalling and environmental changes activate molecular responses inside cells that can lead to corresponding changes in intracellular temperature gradients. The ability to measure intracellular temperature precisely would therefore provide an invaluable tool for cellular biophysicists, potentially allowing cellular behaviour and characteristics to be manipulated by controlling the temperature within, or close to, cellular organelles.

Kucsko and co-workers' technique could also open up many other intriguing topics for research, including the thermal modulation of immune responses[6], molecular mechanisms of therapeutic tissue preservation induced by local cooling[7], the role of subcellular temperature gradients in cell function[8], and cell resistance to hyperthermia treatment[9] (deliberately induced elevated body temperature, used, for example, as anticancer therapy). When it comes to measuring temperature, it may be that diamonds are a scientist's best friend. ■

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CANCER

Angiogenic awakening

Metastatic tumour cells often remain dormant for years. New findings suggest that endothelial cells lining blood vessels have a central role in regulating the transition from dormancy to metastatic growth.

NETA EREZ

The primary cause of cancer-associated deaths is the metastasis of a tumour to distant organs. Advanced metastatic cancers are mostly incurable, and available therapies can only prolong life to a limited extent. In many tumour types there is a long lag between the arrival of cancerous cells at distant locations and their colonization of an organ, such that the formation of clinically evident metastases can take months to decades. This prolonged dormancy suggests that disseminated tumour cells must overcome growth-inhibiting signals from their new local environment in order to take over the metastatic organ[1,2]. The role of the microenvironment in supporting tumour growth at the primary site is well documented[3], but the role of the metastatic microenvironment

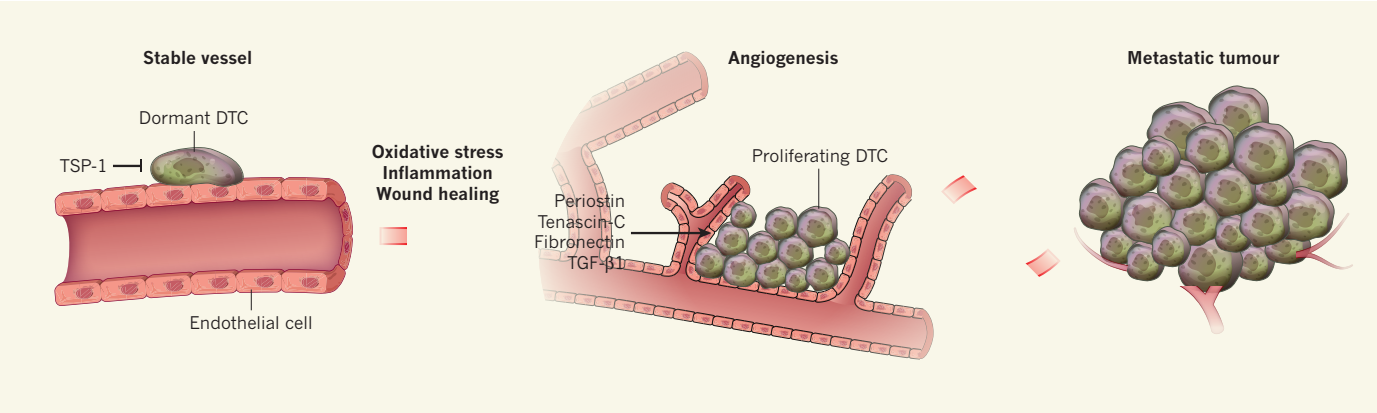

Figure 1 | Instigation of angiogenesis enables tumour-cell exit from dormancy. Ghajar *et al.*[4] show that expression of the protein TSP-1 by endothelial cells in stable blood vessels inhibits the proliferation of disseminated tumour cells (DTCs) residing on the blood vessels. By contrast, DTC proliferation is accelerated at the sprouting tips of new vessels, in part by endothelial-cell secretion of periostin, tenascin-C, fibronectin and TGF-β1. Instigation of angiogenesis (the formation of new blood vessels), for example following oxidative stress, inflammation or wound healing, at a site to which tumour cells have metastasized but are dormant, might thus awaken these cells and enable the formation of metastatic tumours. Proliferating tumour cells will express pro-angiogenic signals that feed back to amplify this process.

and the molecular interactions between disseminated cancer cells and stromal cells at the metastatic organ are poorly characterized. Writing in *Nature Cell Biology*, Ghajar *et al.*[4] report a mechanism by which the endothelial cells that line blood vessels regulate the dormancy of disseminated breast cancer cells*.

Using a mouse model of human breast-cancer metastasis, the authors show that dormant disseminated tumour cells (DTCs) reside on the endothelium of the microvasculature in the lung, bone marrow and brain, which are common metastatic destinations of breast cancer. An *in vitro* three-dimensional model of microvasculature confirmed that this perivascular location of tumour cells is responsible for maintaining their quiescent state, and identified the protein thrombospondin-1 (TSP-1), secreted by endothelial cells, as a suppressor of tumour-cell growth.

Remarkably, this growth-suppressive microenvironment was found only around stable microvascular endothelium; the sprouting tips of newly forming vessels actually had an opposite, growth-accelerating effect on tumour cells. Unlike stable vessels, these growing tips were characterized by enhanced expression of the proteins periostin, tenascin-C, fibronectin and active tumour growth factor-β1 (TGF-β1), which have all previously been implicated in the formation of the metastatic niche[5–7] (Fig. 1).

These findings suggest that the vascular endothelium is an active participant in the formation of a growth-permissive microenvironment. The growth-accelerating effect could be seen *in vitro*: reducing the number of endothelial tips by targeting Notch1, a protein that regulates vessel sprouting, suppressed the proliferation of breast-tumour cells in the microvasculature models. And when the authors injected the tumour cells into zebrafish that were genetically engineered to have excessive microvascular sprouting, they observed enhanced proliferation of the cells adjacent to new blood-vessel tips.

Angiogenesis — the growth of new blood vessels — is crucial to the ability of tumours and metastases to thrive[8,9]. Ghajar and colleagues' findings add a fascinating layer to this story by suggesting that metastatic propagation may rely on the induction of new vascular growth not only because such growth supports nutrient and oxygen supply to micrometastases, but also because it enables the exit of DTCs from dormancy.

But can solitary DTCs exhibit such pro-angiogenic signalling? Dormant tumours are metabolically active and sustain a balance between angiogenic inhibitors and stimulators, remaining dormant until this balance is shifted towards the stimulation of angiogenesis[8,9]. However, it is not clear whether single dormant DTCs can induce angiogenesis. Most previous studies on angiogenesis and tumour dormancy have focused on how tumour-cell characteristics determine the nature of the tumour vasculature. But the present findings imply that, in some cases, the order of events may be reversed, such that the awakening of the vasculature precedes metastatic growth and is independent of pro-angiogenic signalling from dormant DTCs.

In healthy adult tissues, the formation of blood vessels is a relatively infrequent event that is only triggered when certain signals lead to an increase in angiogenic activators — for example, following tissue-damaging events such as oxidative stress, inflammation or wound healing. If such events occurred in an organ in which DTCs reside, the resulting instigation of new vascular growth might inadvertently switch the balance from growth-suppressive to growth-accelerating signals and thereby facilitate the exit of DTCs from dormancy. Thus, escape from dormancy may be, in some cases, secondary to the induction of angiogenesis at the metastatic site (Fig. 1).

The ability of tumour cells to exit dormancy and proliferate seems to be the rate-limiting step of metastasis. Ghajar and colleagues' results emphasize that to confront metastasis in a particular organ, the interactions of tumour cells with the microenvironment of that specific organ need to be elucidated.

Numerous studies have previously demonstrated the central role of innate and adaptive immune cells in the formation of a permissive metastatic environment[1,2], and it is likely that immune cells and other cells at the metastatic site function together with the signals from endothelial cells to regulate tumour-cell dormancy. Ghajar *et al.* used immunodeficient mice in their experiments, but future studies in animal models with intact immunity may shed light on such interactions. Expanding our understanding of the early stages of metastatic growth will pave the way to new targeted therapeutics aimed at preventing, rather than trying to cure, metastatic disease. ■

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