MEDICAL RESEARCH

Neighbourhood deaths switch cancer subtype

How the same type of cell can form different kinds of tumour isn't always clear. The discovery that cancer subtype in mice is influenced by the type of cell death occurring in the microenvironment provides some insight. SEE ARTICLE P.69

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The characteristics used to classify tumours, such as the appearance of cancer cells under a microscope, usually reflect the type of cell from which the cancer originated. Yet sometimes the same type of cell can give rise to cancers that are substantially different in appearance and prognosis. The mechanisms that dictate this type of diversity in cancer development are mainly unknown. Seehawer *et al.*[1] reveal on page 69 that the same type of cell can give rise to different types of cancer depending on the sort of cell death that occurs nearby in the tumour microenvironment. This suggests that nearby injury or damage can affect the identity of a cancer.

Human tumour samples are classified in the clinic using a microscope-based technique called histology to assess the shape and form of tumour cells. A prognosis is determined and treatment decisions are made on the basis of this classification. This approach assumes that cancer cells' appearance reflects that of their cell of origin.

A key mechanism that enables tumour cells to retain the characteristics of their founder cell is the formation of heritable types of alteration, known as epigenetic changes. These are chemical modifications, such as the attachment of methyl groups, that are made to DNA and to the histone proteins that associate with it to form chromatin. Epigenetic modifications affect chromatin structure and can have long-term effects on gene expression without changing the genome sequence.

Differences between particular subtypes of tumour can arise if tumours originate from different types of cell residing in normal tissue[2,3]. But in some cases, the same type of cell can give rise to two different tumour subtypes. One explanation for such a divergence is the presence of mutations that affect cellular appearance[4]. Yet the mechanisms that underlie diversity in cancer subtype are mainly unknown, which is important medically because tumour identity is linked to prognosis and treatment options.

Liver cancer is the second-highest cause of cancer mortality globally[5], and two common histologically distinguishable subtypes are called hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC). Originally, it was thought that differentiated hepatocytes, the main type of cell in the liver, give rise to HCC, and that bile-duct cells (also called cholangiocytes) in the liver give rise to ICC. Yet studies in mice indicate that both HCC and ICC can arise from hepatocytes[6,7]. But how can the same type of cell form two different tumour subtypes that have striking differences in form and progression?

Seehawer and colleagues made a fortuitous discovery when generating liver cancer in mice by the *in vivo* delivery of identical cancer-promoting genes. The animals developed either HCC or ICC, depending on whether the gene-delivery technique was tail-vein injection or electroporation, respectively (Fig. 1). The authors recognized that investigating this observation might shed light on a fundamental aspect of cancer development.

Using lineage-tracing experiments to track the cells of origin for the tumours that arose, the authors found that HCCs and ICCs were both derived from cells called differentiated hepatocytes. Seehawer *et al.* did not find any striking differences in the mutational profiles of the protein-coding DNA sequence in HCCs and ICCs. The authors sampled liver tissue immediately after gene delivery to assess whether the gene-delivery method caused any differences in the tumour microenvironment. Tail-vein injection, the gene-transfer method associated with HCC formation, caused some hepatocytes in the surrounding region of tumour development to undergo a type of cell death called apoptosis, whereas electroporation, associated with ICC formation, caused the neighbouring cells to undergo a type of cell death termed necroptosis.

The experimental introduction of cancer-promoting genes into hepatocytes usually generates HCC as the default, rather than ICC. The authors therefore investigated whether necroptosis influenced ICC formation. They found that if necroptosis was induced after apoptosis occurred in their experimental set-up, it resulted in ICC. Conversely, the suppression of necroptosis, either by drugs or by the presence of certain mutations, favoured HCC formation. These results are consistent with a model in which necroptosis favours the formation of ICC rather than HCC, and suggest that a switch in cancer development towards ICC

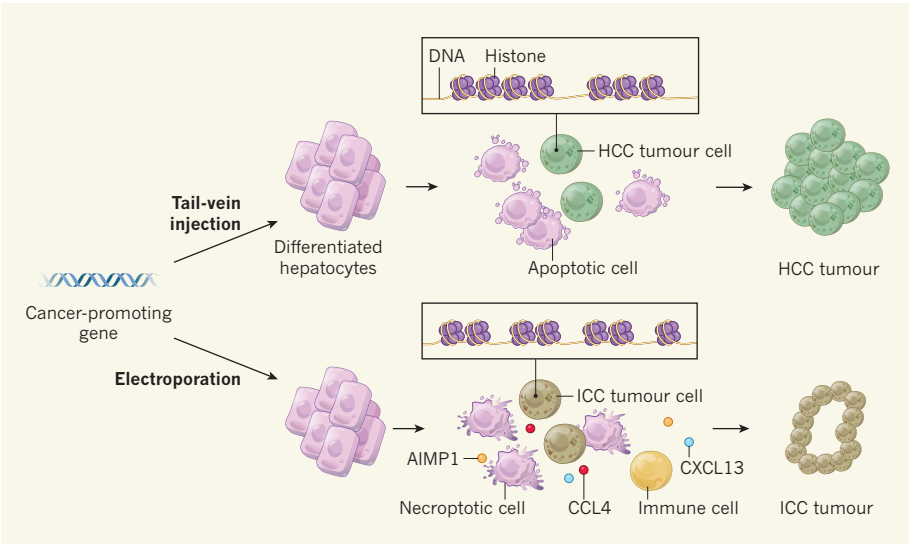

Figure 1 | Nearby cell death influences cancer subtype. Seehawer *et al.*[1] used two techniques (tail-vein injection or electroporation) to transfer the same cancer-promoting genes into differentiated hepatocytes (the main liver cell type) in mice. If tail-vein injection was used, some cells in the microenvironment of the developing tumour underwent a form of cell death called apoptosis, and the tumour subtype that arose was hepatocellular carcinoma (HCC). If electroporation was used, a form of cell death called necroptosis occurred in the microenvironment. Necroptosis was associated with high levels of immune-signalling molecules, including AIMP1, CCL4 and CXCL13, and this form of cell death is generally associated with inflammation and the presence of immune cells. The tumour subtype that arose in this context is called intrahepatic cholangiocarcinoma (ICC). The authors report that there were differences between HCC and ICC cells in the structure of the complex of histone proteins and DNA called chromatin. Such differences can have a long-term effect on gene expression. If cell-death processes affect signalling pathways in nearby tumour-initiating cells in a way that influences chromatin, this might explain the mystery of how the same type of cell can give rise to tumour subtypes that have different appearances and prognoses in humans.

requires a signalling input that is influenced by necroptosis.

Necroptosis is a more inflammatory form of cell death than apoptosis. The authors analysed immune-signalling molecules called cytokines in the livers of mice given the cancer-inducing treatments, and observed differences in the cytokine pattern depending on whether HCC or ICC developed. For example, they noted an increase in the expression of cytokines AIMP1, CCL4 and CXCL13 associated with ICC, compared with their levels in HCC. This raised the question of whether necroptosis-associated inflammation might induce epigenetic changes in hepatocytes that are poised to become cancer cells. The authors found differences in chromatin structure in selected regions of the genome between the two cancer subtypes, although when and how these differences arose is unknown.

Seehawer and colleagues also observed that the transcription-factor protein TBX3 was more highly expressed in HCC than in ICC, whereas the transcription factor PRDM5 had the opposite pattern of higher expression in ICC than in HCC. These expression differences were associated with epigenetic differences in the chromatin structure of the genes encoding these proteins. Remarkably, when the authors analysed samples of human HCC or ICC, they also observed the same pattern of higher TBX3 expression in HCC than in ICC, and higher PRDM5 expression in ICC than in HCC.

Altogether, the evidence suggests that the cell-death conditions prevailing in the liver at the earliest stages of tumour formation might account for the formation of these two different tumour subtypes. Early events in tumour formation are long over by the time a biopsy sample is taken from a human liver; this mouse study could help to illuminate key events that shape the initial steps of tumour formation and result in different cancer identities.

The study provides strong evidence that the tumour microenvironment provides yet-to-be identified signals that can impart long-lasting changes to the fate of cells poised to form cancer cells. Perhaps cytokines might be the drivers of this cancer-subtype switch. If so, the release of such cytokines because of tissue damage or disease might shape the identity of a cancer that is starting to form nearby.

In Seehawer and colleagues' experimental system, the cancer-subtype switch occurred after the acquisition of cancer-promoting genes. However, in human cancers, a different sequence of events is often noted that is thought to occur before the acquisition of cancer-promoting genetic changes. In this scenario, an increased risk of cancer is associated with a process called metaplasia, in which one type of differentiated cell reversibly switches to a different type of differentiated cell. How a predisposition to malignancy arises because of metaplasia is unknown.

It has been reported[8] that the abnormal accumulation of bile-duct-like structures in the livers of mice that have chronic liver disease is not due to the proliferation of bile-duct cells, as was previously thought, but that these structures arise from hepatocytes, and that this therefore constitutes a form of metaplasia. Interestingly, a similar pattern of growth of bile-duct-like cells is often observed in human livers[8,9]. Although this is not usually considered as a form of metaplasia, it is associated with an increase in the risk of liver cancer[9]. Could the switch between HCC and ICC in mice be similar to the process that occurs in liver metaplasia in humans? If it is, Seehawer and colleagues' work might provide insight into how metaplasia increases cancer risk. ■

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IMMUNOLOGY

Put to sleep by immune cells

The sleep disorder narcolepsy is linked to immune-system genes and is caused by the loss of neurons that express the protein hypocretin. Hypocretin-targeting immune cells have now been found in people with narcolepsy. SEE ARTICLE P.63

ROLAND S. LIBLAU

The events that lead to the sleep disorder narcolepsy are a long-standing mystery. On page 63, Latorre *et al.*[1] reveal that people with narcolepsy have unusually high levels of a type of immune cell called a T cell, which targets proteins normally present in neurons in the brain. This finding raises the question of whether narcolepsy arises because T cells unleash an autoimmune response against neurons that are important for sleep regulation.

Narcolepsy affects around 1 in 2,000 people[2]. The symptoms usually begin in adolescence or early adulthood, and include daytime sleepiness and, in some cases, cataplexy — sudden muscle weakness during wakefulness that causes falls. A small population of neurons in the brain produces a protein called hypocretin, which controls sleep–wake cycles[3], and narcolepsy-like symptoms occur in animals that have defects in genes required for the production of or response to hypocretin[4]. Narcolepsy type 2 is associated with daytime sleepiness, and this can progress to narcolepsy type 1, which is characterized by sleepiness and cataplexy. People with narcolepsy type 1 have abnormally low numbers of hypocretin-producing neurons[5].

Hypocretin levels in the cerebrospinal fluid that bathes the brain and spinal cord can be measured to help diagnose[6] narcolepsy type 1, and such tests provide a way of indirectly monitoring the loss of hypocretin-producing neurons over time. The trajectory of this neuronal loss remains to be fully understood, but can take months or years.

Studies of human genetics have implicated immune-system genes in narcolepsy. Yet whether the immune system contributes to the demise of hypocretin-producing neurons, and if so, how, was unknown. HLA genes encode proteins that can present protein fragments called antigens to T cells, and this interaction can trigger an immune response against cells that contain the specific antigen. Autoimmune diseases are often associated with HLA genes[7]. A version of one such gene, called *HLA-DQB1*06:02*, is present in more than 98% of people with narcolepsy[8], but it is found in only 15–30% of the general population, depending on ethnicity[8,9]. Moreover, previous reports[10,11] suggest that antibodies targeting neuronal proteins are present at a higher than usual frequency in people with narcolepsy.

"Do T cells that target neuronal proteins other than hypocretin have a role in narcolepsy?"

Latorre and colleagues used various techniques to identify human T cells that recognize specific antigens. The authors tested whether T cells that recognize antigens from hypocretin were present in blood samples