Oncofetal reprogramming in tumour development and progression

Ankur Sharma, Camille Blériot, Jennifer Currenti and Florent Ginhoux

Abstract | Embryonic development is characterized by rapidly dividing cells, cellular plasticity and a highly vascular microenvironment. These features are similar to those of tumour tissue, in that malignant cells are characterized by their ability to proliferate and exhibit cellular plasticity. The tumour microenvironment also often includes immunosuppressive features. Reciprocal communication between various cellular subpopulations enables fetal and tumour tissues to proliferate, migrate and escape immune responses. Fetal-like reprogramming has been demonstrated in the tumour microenvironment, indicating extraordinary cellular plasticity and bringing an additional layer of cellular heterogeneity. More importantly, some of these features are also present during inflammation. This Perspective discusses the similarity between embryogenesis, inflammation and tumorigenesis, and describes the mechanisms of oncofetal reprogramming that enable tumour cells to escape from immune responses, promoting tumour growth and metastasis.

Tumour growth has been compared to embryonic development owing to its features of cellular plasticity and tissue expansion[1,2]. It is important to note that epithelial-to-mesenchymal transition, a fundamental process in the initiation and metastasis of some (although not all) tumour types, was first characterized during embryogenesis[3,4]. Furthermore, the high proliferative capacity of some tumour cells recalls that of embryonic stem cells, which can give rise to all tissues of an embryo. However, unlike embryonic stem cells, tumour cells bear oncogenic mutations that render them capable of unlimited replicative potential[5]. Although some comparisons have been made between embryonic development and tumorigenesis in the existing concept of oncofetal antigens, which refers to the re-expression of fetal genes and/or proteins by malignant cells, similarities between the tumour microenvironment and the developing fetus remain to be explored in detail. In this Perspective, we extend the conceptual framework underlying oncofetal reprogramming of malignant cells to include the fetal-like reprogramming of immune and stromal cells in the tumour microenvironment. We term this expanded concept the 'oncofetal ecosystem' (FIG. 1).

The tumour microenvironment plays a key role in supporting the colonization, growth and progression of tumour cells[6], and recent advances in single-cell analyses have provided an unprecedentedly granular view of the tumour microenvironment[7]. These methods have led to the identification of heterogeneous tumour-specific fibroblasts, endothelial cells and other immune cells embedded in the tumour extracellular matrix[7–12]. Reciprocal communication between these subpopulations enables tumour cells to proliferate, migrate and escape immune responses[13,14]. Each of these elements contributes to tumour biology; therefore, an enhanced understanding of cellular interactions occurring within the tumour microenvironment is essential for designing more-effective clinical treatments, given that targeting fetal-like cells could influence treatment responses. More importantly, some of these fetal-like features have been observed during tissue regeneration[15], and thereby indicate links between embryogenesis, regeneration and/or inflammation, and tumorigenesis (FIG. 1).

The well-established link between regeneration and/or inflammation and tumorigenesis led to tumours being described as "wounds that do not heal"[16]. In wound healing, a complex interplay of positive-feedback and negative-feedback interactions[17] enables inflammation, regeneration and remodelling to be initiated and resolved in a structured manner[18]. By contrast, in tumorigenesis, the lack of negative-feedback mechanisms can lead to persistent inflammation, cell persistence or proliferation, and fibrosis in some cancers such as Hodgkin lymphoma[19].

Chronic inflammation increases the risk of cancer and promotes tumour progression[20]. Chronic viral infections and cirrhosis increase the risk of hepatocellular carcinoma (HCC)[21], reflux can lead to Barrett oesophagus, which is a risk factor for oesophageal adenocarcinoma[22], schistosomiasis increases the risk of bladder and colon carcinomas[23], Crohn's disease and ulcerative colitis are risk factors for colorectal cancer (CRC)[24], and chronic *Helicobacter pylori* infection predisposes individuals to develop stomach cancer[25]. Long-term exposure to inflammatory mediators is thought to promote tumorigenesis through mutagenesis, angiogenesis, oncogene activation and increased cell proliferation[26]. Furthermore, chronic inflammation promotes epithelial-to-mesenchymal transition, which is linked to tumour invasiveness and metastatic potential[27]. This key role of inflammation in tumorigenesis is accentuated by its status as a hallmark of cancer[28]. In addition, some aspects of inflammation are involved in embryogenesis, and some elements of embryogenesis are present during the inflammatory processes inherent in tissue repair and regeneration. For example, although epithelial-to-mesenchymal transition is primarily associated with chronic inflammation, it is also essential for many stages of embryogenesis[3,4] and tumour development[29], as well as for tissue repair and regeneration[30] and wound healing[31]. In inflammatory skin diseases, fetal-like cells have been observed[32,33], suggesting that tissue remodelling following damage and inflammation relies on mechanisms that are reflective of embryogenesis.

Newer hallmarks of cancer include considerable phenotypic plasticity and non-mutational epigenetic reprogramming[28].

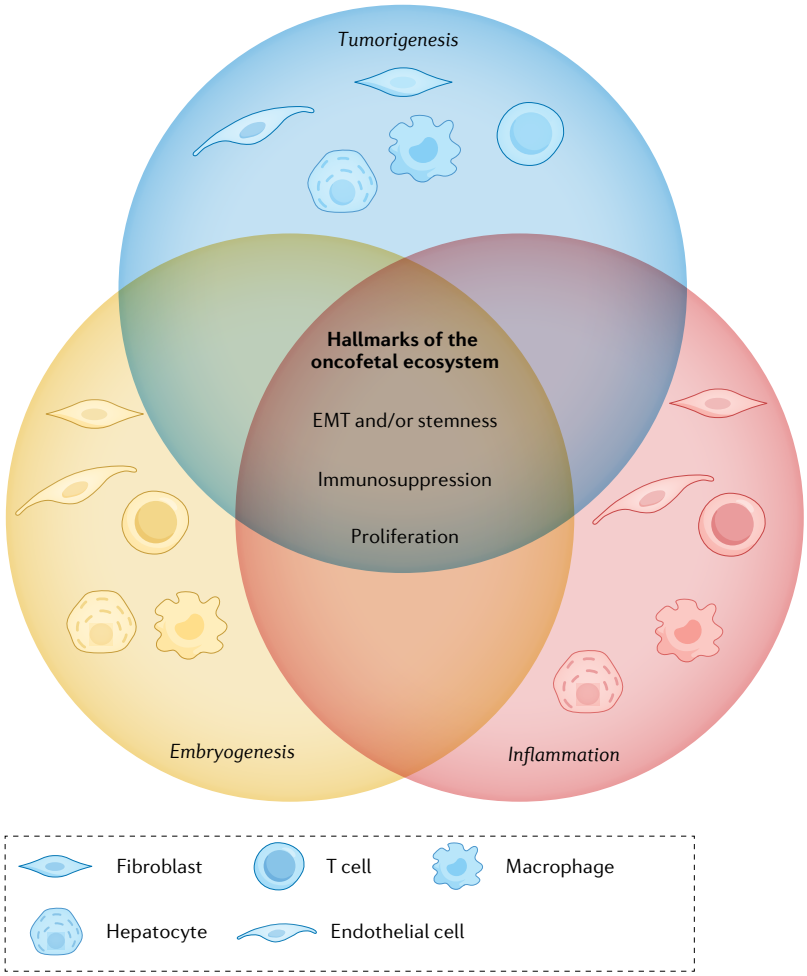

Fig. 1 | **Similarities between embryogenesis, inflammation and tumorigenesis.** The oncofetal ecosystem is characterized by the reappearance of fetal-like cells in the tumour microenvironment. We hypothesize that re-expression of a fetal-like state is an evolutionary adaptation that supports regeneration and/or inflammation (for example, during cirrhosis). The hallmarks of oncofetal reprogramming are proliferation, epithelial-to-mesenchymal transition (EMT) and/or stemness, and immunosuppression, which collectively lead to tumour growth and development.

These two factors also play important roles in embryonic development and inflammation[34]. Furthermore, the tumour microenvironment plays an important role in tumorigenesis[6,14], and the presence of inflammatory cells in the tumour microenvironment has been associated with tumour-promoting properties[35].

In this Perspective, we introduce the idea of the oncofetal ecosystem. Specifically, we outline the similarities between embryogenesis, inflammation and tumorigenesis, and describe emerging evidence of oncofetal reprogramming in non-cancerous cells of the tumour microenvironment, including the influence of epigenetic states and cell-to-cell communication. The possibility of oncofetal reprogramming in paediatric tumours and relevance of these findings to current and developing treatment strategies are also explored.

Historical overview

Oncofetal proteins and/or antigens. Tumorigenesis has been linked with embryogenesis since at least 1908 when tumours were suggested to be an abnormal continuation of embryonic cell formation[36]. Several later permutations of this idea maintained the link with fetal development. In the 1960s, shared fetal and tumour antigens were identified in cancers of the liver and digestive organs, termed 'carcinoembryonic antigens' or 'oncofetal antigens'[37–39]. Since then, oncofetal antigens have been identified in several tumour types, and a few are now well established as clinical biomarkers, notably for early cancer diagnosis[40–44] (FIG. 2).

α-Fetoprotein (AFP) is one of the best-characterized oncofetal antigens[45,46]. In adults with chronic viral infections (such as hepatitis B and hepatitis C), AFP levels are increased, and are even higher in individuals with these infections who also have HCC[47]. Indeed, elevated levels of AFP can be detected in the blood of patients with various hepatic tumours[48], and blood AFP levels are considered a reliable diagnostic biomarker for these tumour types[49–51].

Other diagnostic markers that can be detected in human blood include carcinoembryonic antigen (CEA, a marker of CRC[52–54]) and cancer antigen 125 (CA125, also known as mucin 16, a marker of breast, endometrial and ovarian cancers[55–57]). Interestingly, CA125 levels can distinguish ovarian tumours from endometrial tumours and have implications for treatment response and risk of relapse in patients with ovarian cancer[58]. Importantly, CA125 is also a relevant blood-based biomarker for familial ovarian cancer in carriers of *BRCA1* and *BRCA2* mutations[59–61]. Furthermore, oncofetal antigens are not limited only to fluid biomarkers; the transcription factor Sal-like protein 4 (SALL4), a nuclear factor that is active during embryonic development and that is crucial for the maintenance of stem cell pluripotency, is an oncofetal antigen detectable in HCC tumours[62], although it is not currently used as a diagnostic or prognostic marker. Other transcription factors, such as FOXM1 (REF.[63]) and insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1)[64,65], have also been associated with oncofetal properties.

Oncofetal genes and RNAs. Expression of *LIN28B* (which encodes LIN-28 homologue B) is suggested to be an oncofetal marker for stem cell-like circulating tumour cells because levels of its mRNA correlate with recurrence of HCC after hepatectomy[66].Several long non-coding RNAs (lncRNAs) and microRNAs (miRNAs) have been identified as associated with both embryonic development and tumorigenesis, but are inactive in normal homeostasis[67,68]. Interestingly, some of these miRNAs and lncRNAs are regulated by *TP53* (which encodes tumour suppressor p53); dysfunction of p53, often through loss-of-function mutations, is observed in a large proportion of human cancers[69]. The oncofetal lncRNA H19 is produced in fetal tissues and in several tumours, including CRC and HCC[70]. In embryos, H19 promotes cell differentiation, whereas in adults H19 is rarely detected, except in malignant tumour cells[70]. An interplay between p53 and H19 has been identified in bladder cancer, whereby miR-675 (the mature product of H19) is an important inhibitor of p53 (REF.[71]).

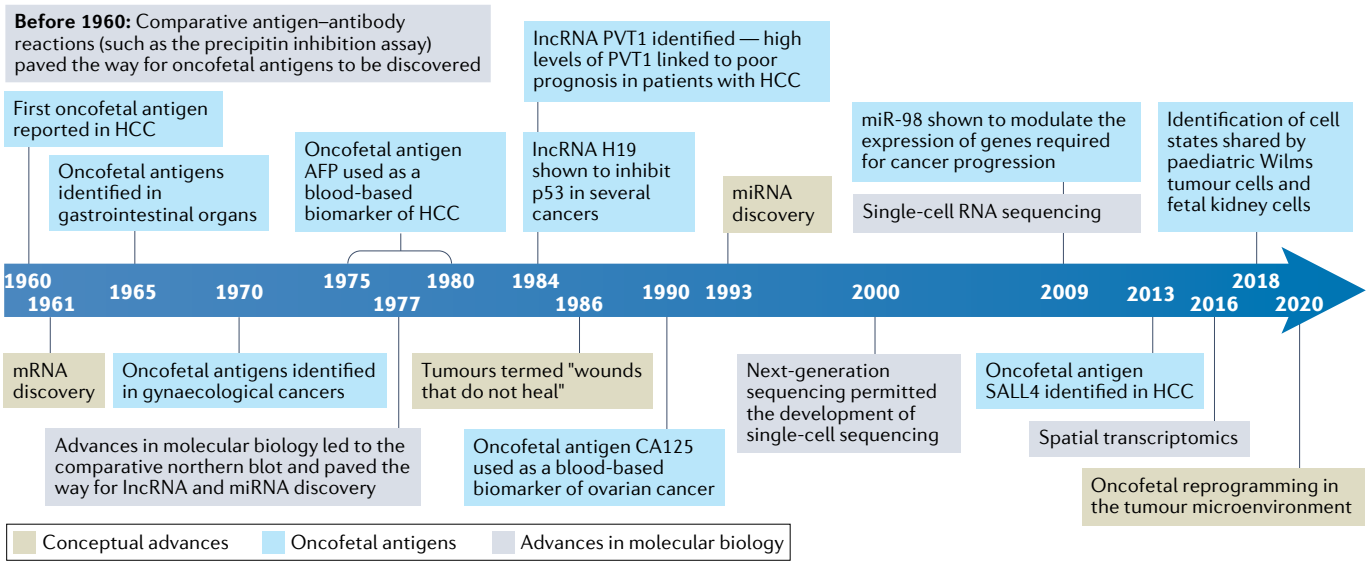

Fig. 2 | **Timeline of developments in the history of oncofetal reprogramming.** The early era of the oncofetal concept was dominated by discoveries from biochemical experiments. In the 1960s, oncofetal antigens were discovered in hepatocellular carcinoma (HCC)[37,39], which was followed by similar discoveries in other gastrointestinal cancers[38] and breast cancer[41]. Within a decade, α-fetoprotein (AFP) had gained prominence as a blood-based biomarker for the detection of primary liver cancer[51]. Advances in molecular biology led to the identification of long non-coding RNAs (lncRNAs) active in fetal and tumour environments[178], concomitant with the description of tumours as "wounds that do not heal"[16]. Later, the oncofetal antigen cancer antigen 125 (CA125) entered use in the clinic as a diagnostic tool to detect cancer in patients with endometriosis[55]. The discovery of microRNAs[179] led to the identification of several with oncofetal properties[83]. Subsequently, the oncofetal gene *SALL4* was linked to HCC[62]. Advances in single-cell genomics, single-cell RNA sequencing[180] and spatial transcriptomics[181] paved the way for the discovery of cell populations shared between fetal tissue and cancer, such as the fetal-like cells identified in Wilms tumour[92] and the similar cell types in the fetal and tumour microenvironments identified by our group that led to the concept of the oncofetal ecosystem[8]. miRNA, microRNA; SALL4, Sal-like protein 4.

In gastric cancer, H19 interacts with p53, probably via a negative-feedback loop, causing partial inactivation of p53 (REF.[72]). Similarly, lncRNA PVT1 and its downstream molecules have low expression in normal tissues[73] and are upregulated in several tumour types, such as cholangiocarcinoma[65], ovarian cancer[74] breast cancer[75], acute myeloid leukaemia[76], Hodgkin lymphoma[77] and paediatric malignant astrocytoma[78]. In patients with HCC, high levels of lncRNA PVT1 were associated with a poor prognosis as a result of their altering the stem cell-like (or fetal-like) properties of tumour cells, promoting their growth[79]. In addition, several lncRNAs have been linked to metabolic switching to glycolysis during tumorigenesis, including PVT1 (REF.[80]) and H19 (REF.[81]). In patients with oral squamous cell carcinoma, for example, H19 is involved in enhancing the glycolysis pathway in cancer-associated fibroblasts (CAFs)[82].

Furthermore, miRNAs of the let-7 family, such as miR-98, modulate the expression of genes with important roles in cancer progression[83]. One such example is IGF2BP1, a target of let-7 that has an oncofetal expression pattern[84]. Another miRNA, miR-17-5p, is upregulated in fetal colorectal tissues and in CRC tumours, and more-advanced tumours express higher levels of miR-17-5p[68].

The oncofetal ecosystem. Evidence collected in the past few decades has highlighted a striking resemblance between fetal development and the growth of several types of tumours, supported by the identification of a catalogue of shared proteins and RNAs in these two convergent contexts.

Although common proteins and RNAs can be identified in both embryos and tumours, tumorigenesis and embryogenesis differ in at least one fundamental aspect: embryonic development is organized and regulated, whereas tumorigenesis is characterized by aberrant and unchecked growth. However, these two processes do share an important characteristic: both embryonic stem cells and tumour cells display immunosuppressive properties that lead to an immune-tolerant microenvironment. For example, mesenchymal progenitors derived from human embryonic stem cells display immunosuppressive properties towards lymphoid cells[85], and findings in cell lines[86] and mouse models[87,88] suggest that cancer stem cells (which have been identified in some but not all tumour types) can exhibit immune evasion. Consequently, growing evidence suggests that the tumour microenvironment is not only of central importance for tumorigenesis[28] but also shares mechanisms and effectors with embryogenesis[8], a point absent from the classic view of oncofetal genes and proteins that applies only to tumour cells.

Thus, we hypothesize that a fetal-like tumour microenvironment plays a role in tumorigenesis. Although both the fetal microenvironment and the tumour microenvironment promote cell proliferation and have immunosuppressive properties, an important difference between these settings is that the embryonic microenvironment possesses mechanisms for the control of cell proliferation that are likely to be missing from the tumour microenvironment. For example, tumour cells transplanted into a mouse embryonic environment showed reduced tumorigenic properties[89,90], an approach later validated in a zebrafish model[91]. These studies support our hypothesis that an oncofetal ecosystem within the tumour microenvironment shares growth-promoting and immunosuppressive properties with its embryonic counterparts, but lacks the regulatory mechanisms present in embryogenesis.

Evidence from single-cell omics

Advances in single-cell genomics, epigenomics and spatial transcriptomics methods could help to confirm and extend the observed similarities between fetal development and tumorigenesis, notably

by identifying cells that share fetal and tumoural identities[8,92] and cell type-specific patterns of oncofetal gene expression[92–94].

These technologies have already been used in paediatric tumours arising from embryonic stem cells. For example, single-cell RNA sequencing analysis led to the identification of cell states shared by paediatric Wilms tumour cells and fetal kidney cells, indicating that oncofetal programmes are activated in childhood renal cancers, although whether such activation occurs as a result of oncofetal reprogramming and/or maturation block is yet to be determined[92,95]. Moreover, paediatric renal cancer can originate as a result of lack of maturation in aberrant fetal cells[92], thereby showing the power of comparative single-cell genomics to study similarities between embryonic development and tumorigenesis. Wilms tumours arose from the premalignant embryonic tissue bed in 14 of 23 paediatric patients (~61%)[95], providing evidence that precancerous clonal expansion can be the source of these tumours, as has also been observed in adult renal cancers. However, it is important to note that although some paediatric tumours (such as lymphoblastic leukaemia and Wilms tumour) are known to involve a maturation block that keeps tumour cells in an oncofetal state[96], whether a component of this oncofetal reprogramming involves the tumour microenvironment as it does in adult tumours[8] remains to be determined.

A separate single-cell RNA sequencing study that profiled cell populations in the developing pons and forebrain[97] aimed to clarify the association of these cells with embryonal tumours with multilayered rosettes (ETMR), which are lethal paediatric brain tumours. The results of this study demonstrated similarities, referred to as an 'early neural progenitor-like cell state', between ETMR tumours and the fetal brain as well as shared activation of the Tweety homologue 1 (TTYH1), *LIN28A* and DNA (cytosine-5)-methyltransferase 3B (DNMT3B) pathways. Two reports from 2021 demonstrated that the presence of chemotherapeutic drugs caused malignant cells to adopt a distinct MYC-dependent transcriptional programme that resulted in a suspended developmental state resembling embryonic diapause[98,99]. These studies further strengthen the notion that tumour cells can undergo embryonic-like reprogramming that drives their survival and proliferation.

Multidimensional omics analyses have revealed similarities between fetal and tumoural contexts that provide an opportunity to identify fetal-like characteristics and oncofetal reprogramming in tumour cells. However, one could argue that this approach, even if highly discriminative, does not prove that oncofetal reprogramming occurs only in tumour cells. The available studies have not yet explored whether oncofetal reprogramming occurs in non-malignant cells of the tumour microenvironment. However, as the survival of tumour cells involves interactions with the tumour microenvironment[100,101], we hypothesize that oncofetal reprogramming of the tumour microenvironment is important in tumorigenesis.

Oncofetal reprogramming

Reports from the past 2 years have highlighted important implications of oncofetal reprogramming in non-malignant cells within the tumour microenvironment, including tumour-associated macrophages (TAMs)[8,102], endothelial cells[8,103], fibroblasts[82,104] and T cells[105,106]. For example, paracrine signalling by local cell populations is involved in the initial transformation process[107] as well as in tumour progression[100,108,109]. Similarly, the epithelial regeneration occurring in wound healing is driven not only by inflammation but also by crosstalk with the immune microenvironment[18], highlighting potential roles of the local tissue microenvironment in both inflammation and tumorigenesis. In this section, we mostly focus on macrophages and endothelial cells. Other cell types within the tumour microenvironment that might also undergo oncofetal reprogramming have been less well described.

Tumour-associated macrophages.

TAMs have long been associated with immunosuppression, and their increased abundance is well described to be associated with worse prognosis[101,110]. Furthermore, TAMs are important in tumour-related inflammation[111]. Over the past decade, our understanding of macrophage ontogeny has been considerably clarified by observations indicating that tissue-resident macrophages in adults are mostly derived from embryonic progenitors that seed the tissue before birth[112–115], although some unique tissue-resident macrophage populations, such as those inhabiting the gut lamina propria, are constantly replenished by adult circulating monocytes[116–118].

By contrast, tumour development induces massive recruitment of circulating monocytes, which give rise to TAMs[119,120]. Thus, any given tumour contains ontogenically distinct TAM populations: those derived from embryonic tissue-resident macrophages that inhabited the tissues before the tumour arose, and those arising from recruited monocytes. Interestingly, in a mouse model of pancreatic adenocarcinoma, these embryonic and monocytic macrophage populations act differently in the tumour microenvironment: embryonic TAMs assume a profibrotic role, whereas infiltrating monocytic TAMs mostly orchestrate immune responses[120]. Whether this difference in function is due to the intrinsic differences in their origin and/or to the different locations in the tumour microenvironment where they are remains to be further investigated.

Considering the dual origins (that is, embryonic or monocyte derived) of macrophages and their potential implications for the function of adult cells, many studies have dissected the origin of macrophages in multiple organs, healthy and/or diseased, and have attempted to identify markers that characterize their embryonic versus monocytic origins[121–125]. In studies that compared fetal liver with HCC tumour tissues, we and others have observed that fetal liver macrophages express *FOLR2* (which encodes folate receptor 2 (FOLR2)), a feature that is also observed in one subpopulation of TAMs[8,102,126]. Interestingly, in a fate-mapping mouse model that enables the tracking of monocyte-derived macrophages, we observed that this FOLR2$^+$ macrophage population had a dual origin: some of these cells were derived from embryonic precursors, whereas others were derived from adult bone marrow precursors[8]. These observations support the ability of adult macrophages of monocytic origin to undergo embryonic reprogramming and acquire a fetal-like identity. In addition, whereas embryonic FOLR2$^+$ macrophages are present in mouse and human tissues such as the liver under homeostatic conditions[127], FOLR2$^+$ macrophages have also been observed in non-hepatic tumours, such as breast cancer[128]. Whether this macrophage population is derived from embryonic precursors self-maintained in the cancer or from recruited monocytes that are reprogrammed into embryonic-like cells in the tumour microenvironment remains to be determined. Interestingly, recruited monocytes that are reprogrammed into embryonic-like cells are not a tumour-specific phenomenon, as FOLR2$^+$ macrophages have also been observed in cirrhosis[15]. Therefore, investigations into the occurrence of fetal-like reprogramming

of monocytes outside the tumour microenvironment would be of interest.

TAMs also express fetal isoforms of extracellular matrix proteins. In several tumours, migration-stimulating factor (MSF; an oncofetal isoform of fibronectin) is produced by fibroblasts, tumour cells, tumour-associated vascular endothelial cells and fetal skin keratinocytes[129]. MSF is thought to contribute to tumorigenesis through its promotion of angiogenesis and hyaluronan biosynthesis, and stimulation of tumour cell motility[130,131]. A different study that identified production of MSF by in vitro tumour-conditioned macrophages as well as TAMs in ovarian tumours also highlighted the role of MSF produced by TAMs in stimulating tumour cell migration[132]. Collectively, these studies reveal oncofetal reprogramming of TAMs in the tumour microenvironment and their possible roles in supporting tumorigenesis. However, although TAMs do undergo oncofetal reprogramming, the cause of this phenomenon is not fully known.

Endothelial cells. The imprinting of macrophages by their microenvironment has been extensively characterized in the healthy liver, where depletion of native macrophages induces recruitment of naive monocytes that undergo quick and profound rewiring mainly orchestrated by endothelial cells to acquire their resident macrophage identity[133–135]. Accordingly, in tumour samples from patients with HCC, we specifically identified a population of endothelial cells that express *PLVAP* (encoding plasmalemma vesicle-associated protein (PLVAP)). Expression of *PLVAP* is fetal specific and endothelial cell specific, and PLVAP is involved in vascular permeability, leukocyte migration and angiogenesis[136,137]. Importantly, PLVAP$^+$ endothelial cells are largely absent in normal liver but exist in fetal[136,138] and tumour-bearing[8,139] liver. The presence of PLVAP$^+$ endothelial cells within liver tumours suggests the reactivation of embryonic developmental programmes. Of note, another study[15] reported the presence of PLVAP$^+$ endothelial cells in cirrhotic livers and their absence in healthy livers, which argues for a wider role of fetal reprogramming in liver regeneration and disease. Interestingly, PLVAP-deficient mice displayed impaired seeding of tissue-resident macrophages during embryonic development, demonstrating the critical role of PLVAP$^+$ endothelial cells in this process[136]. Further analysis indicated the involvement of canonical Notch–Delta-like protein 4 (DLL4) signalling in interactions between PLVAP$^+$ endothelial cells and FOLR2$^+$ macrophages in the liver tumour microenvironment and in healthy fetal liver[8]. In addition, both PLVAP$^+$ endothelial cells and FOLR2$^+$ macrophages have been reported as novel subpopulations in the gastric tumour microenvironment[140]. These observations suggest that PLVAP$^+$ endothelial cells could induce oncofetal reprogramming of TAMs.

Cancer-associated fibroblasts. One report has identified PLVAP$^+$ hepatic stellate cells in mice[141]; however, whether *PLVAP* is expressed in human liver fibroblasts remains to be investigated. Although the evidence for oncofetal reprogramming of fibroblasts in the tumour microenvironment is not as clear as that for TAMs and endothelial cells, some research that linked fibroblasts with inflammation in the context of liver fibrosis has suggested a potential role for oncofetal reprogramming of CAFs. Fetal fibroblasts have remarkable wound-healing properties and facilitate the process of liver regeneration[142]. Similarly, scar-producing myofibroblasts have been identified in cirrhotic liver but not in healthy liver[15]. CAFs are a major component of the tumour stroma and play a protumorigenic role in breast, prostate and oral cancers[143,144].

Research into CAFs points to their role in tumour initiation, progression and invasion[145,146]. Mechanistic suggestions for their role in glycometabolism revolve around the increased supply of energy to tumour cells, which is mediated by the expression of several lncRNAs, as already discussed[82]. In addition, cell surface expression of MSF has been reported in fibroblasts from patients with breast cancer[147,148]. A fetal-like tumour stroma has been observed to promote the progression of breast cancer, lung cancer, CRC, oral cancer and prostate cancer[129]. Taken together, the results of these studies point to the possibility that fibroblasts in the tumour microenvironment might also undergo oncofetal reprogramming.

Deciphering the tumour micro-environment signals that lead to and sustain oncofetal reprogramming would significantly advance our understanding of oncofetal reprogramming in tumorigenesis. In addition, given the immunosuppressive roles of tumour-specific endothelial cells[149], understanding how these cells are generated and how they interact with immune cells could lead to improved therapeutic applications. Finally, exploring whether other stromal and immune cells of the tumour microenvironment could also drive oncofetal reprogramming or be subject to such reprogramming would be of interest to delineate the oncofetal ecosystem and the crosstalk between its various components (FIG. 3).

Temporal heterogeneity

Tumorigenesis is a stepwise process resulting from the accumulation of a series of genetic and phenotypic changes perhaps facilitated by a fetal-like immunosuppressive microenvironment. However, the landscape and properties of the tumour microenvironment evolve throughout tumorigenesis; for example, a naive monocyte recruited during early tumorigenesis might be exposed to a microenvironment different from that to which a naive monocyte recruited into a late-stage tumour is exposed. In healthy liver, for example, experimental ablation of Kupffer cells (embryonically seeded resident macrophages) induces a massive recruitment of naive monocytes that start to acquire a Kupffer cell-like phenotype within a few hours of arriving in the liver[133,150]. However, these cells' acquisition of the entire native Kupffer cell epigenetic landscape can take a few weeks or even months[133,134]. Similar observations have been made in healthy lungs, where monocyte-derived alveolar macrophages are functionally different from native embryonic macrophages, particularly in terms of trained immunity (the ability of the innate immune system to form immune memory, which provides long-lasting protection against previously seen antigens)[151,152]. Although functional differences might be expected, given the limited antigen exposure of infiltrating cells, they nonetheless imply that ontogeny is important. As in the liver, these functional differences primarily reflect epigenetic modifications that can be hidden by convergent phenotypes, especially when those phenotypes are analysed by low-resolution approaches such as immunochemistry or flow cytometry.

Applying these observations to the tumour microenvironment, we now know that several subpopulations of TAMs with different origins, microenvironments, immune histories and functions coexist, and deciphering their biology requires the integration of these different parameters[115]. Concordantly, the oncofetal reprogramming of myeloid cells (which might also occur at the epigenetic level[133,134,153]) could be suboptimal (meaning that only subsets of fetal-associated genes are expressed) or could affect only one or a few subpopulations of TAMs. Tumour-specific microenvironmental

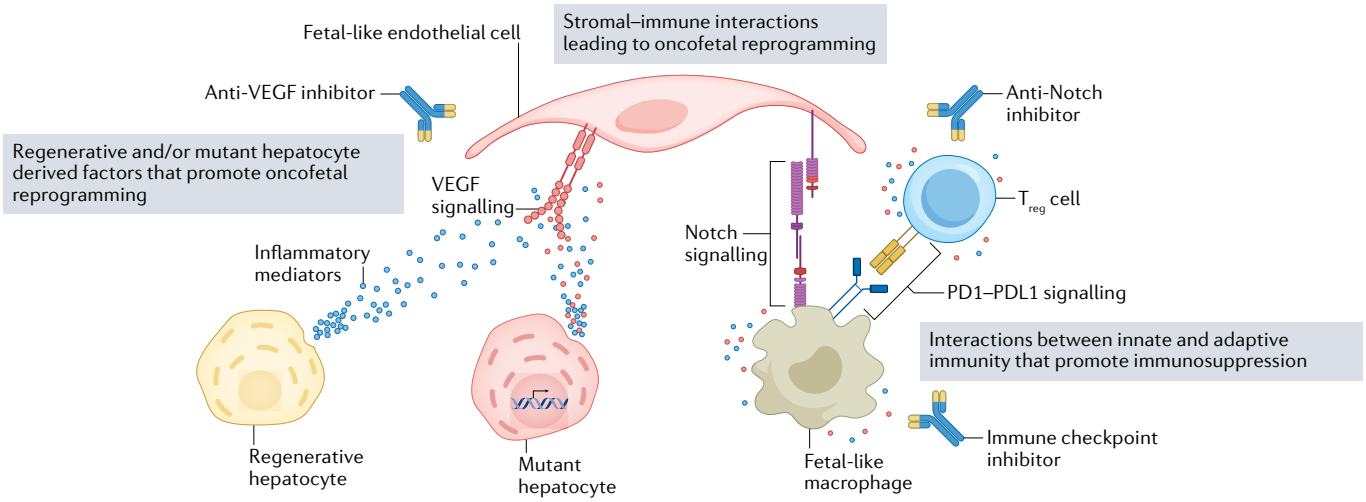

Fig. 3 | **Mechanism of oncofetal reprogramming.** Fetal-like reprogramming of the tissue microenvironment is induced by growth-promoting signals from proliferating cells. During tissue regeneration, these signals are produced by regenerating cells (for example, repairing hepatocytes in individuals with cirrhosis). In individuals with cancer, malignant cells (such as malignant hepatocytes in hepatocellular carcinoma) can produce these signals. These growth-promoting signals promote a proliferative and immunosuppressive tissue microenvironment that can facilitate either regeneration or cancer. We hypothesize that in regeneration these signals are transient and diminish once regeneration is complete, whereas in cancer, mutated cells constantly supply these signals, creating a positive-feedback loop. Importantly, the presence of oncofetal reprogramming within tumours provides multiple avenues for therapeutic targeting of tumour-specific signalling networks by using immune checkpoint inhibitors and antibodies to factors such as VEGF and Notch. T_{reg} cell, regulatory T cell.

conditions could, therefore, be interrogated to reveal these epigenetic programmes, and might potentially explain why one TAM subpopulation with a phenotype indistinguishable from the others becomes tumour promoting or tumour attacking.

The tissue environment clearly affects the epigenetic reprogramming of myeloid cells[153]. Genes such as *IGF2BP1*, *IGF2BP2* and *IGF2BP3* that are regulated by the RNA epigenetic modification N^6-methyladenosine and are normally expressed during embryonic development and downregulated during homeostasis have been shown to be reactivated in tumour cells[154]. Such genes could, therefore, contribute to reprogramming of cells in the tumour microenvironment. Re-expression of embryonic-associated genes is a characteristic of oncofetal reprogramming, and suggests that such phenomena might involve epigenetic events. Another study identified bivalent domains (trimethylated histone H3 Lys4 and trimethylated histone H3 Lys27) in samples of human breast cancers, which the researchers described as a signature of oncofetal epigenetic control[155]. These bivalent domains are reminiscent of those in embryonic stem cells[156,157].

These data raise the fascinating question of whether oncofetal reprogramming has a stochastic or deterministic nature (or both, at least in the context of immune cells, which can have different origins in embryonic and adult tissues). In the stochastic scenario, every monocyte that encounters an oncofetal niche, such as PLVAP$^+$ DLL4$^+$ endothelial cells, will acquire oncofetal programming. In the deterministic scenario, a subpopulation of monocytes with a predetermined epigenetic state might be fated to undergo oncofetal reprogramming. Of note, evidence of such a subpopulation of predetermined circulating monocytes has already been found during the recovery phase of tissue injury, although whether this fate is related to the epigenetic profiles of these cells remains unclear[158]. Furthermore, epigenetic modifications could occur in monocyte progenitors in the bone marrow[159]. Considering that monocytes are continuously recruited during the development of a tumour and the systemic consequences of tumorigenesis for haematopoiesis in the bone marrow, the stochastic and deterministic hypotheses both seem to be valid and should be tested in future experiments.

Spatial heterogeneity. Another important property of tumours is their three-dimensional architecture and heterogeneous cell types. In solid tumours, different tumour cell types are found from the tumour core to the periphery, resulting in a multilayered tumour microenvironment[12,160], whereas in non-solid tumours, tumour cell properties differ within distinct compartments such as the bone marrow and circulation. For instance, in solid tumours, the tumour core is more hypoxic than the peripheral malignant tissue[161]. Whether such zonation also exists for tumour-associated immune cells and how this spatial localization might affect oncofetal reprogramming of these cells remains to be thoroughly investigated. However, PLVAP$^+$ endothelial cells are enriched in the tumour periphery[8]. In addition, various macrophage populations are differentially enriched in the tumour core and periphery, where they play important roles in tumour cell migration[162] and immune evasion[102]. With regard to non-solid tumours, the bone marrow microenvironment contains heterogeneous niches consisting of both haematopoietic stem cells and leukaemic stem cells[163]. Furthermore, leukaemic stem cells can promote remodelling of the adjacent bone marrow microenvironment[164].

Although the spatial dependency of oncofetal reprogramming has been described in the solid tumour microenvironment, comparatively little is known about the spatial dependency of oncofetal reprogramming in non-solid tumours. Emerging transcriptomic approaches will be crucial for tackling this knowledge gap. Indeed, single-cell transcriptomic profiles can now be determined in fixed tissues that conserve the organization of the tumour. Although this technology is still developing, the initial results have been very encouraging[165,166] and will certainly improve our understanding of tumour biology by deconvoluting the functions of individual cells within this

complex environment. Finally, even for primary tumours that initially develop as a result of only one or a few discrete events, advanced disease stages are usually characterized by metastases that have spread to distant tissues[5,167]. For example, the liver is a common site of metastasis for melanoma, breast cancer, pancreatic cancer and colon cancer[168], among other cancers. The tumour microenvironment of primary melanoma is different from that of the liver metastases[169], and whether liver metastases also induce oncofetal reprogramming remains to be comprehensively investigated. As patients usually begin to undergo therapeutic interventions during these late disease stages, an improved understanding of the ongoing interactions specific to primary tumours and metastases are expected to refine the efficacy of treatment.

Paediatric cancers. Paediatric cancers are defined as those arising as consequences of physiological growth and originating from embryonic stem cells[170]. Interestingly, whereas oncofetal reprogramming leads adult HCC[8] and colon cancer[93,94] cells to acquire embryonic states, paediatric tumours often show evidence of impaired cell differentiation or development. For example, the presence of immature hepatocytes in hepatoblastoma or the presence of immature kidney cells in Wilms tumour both result from stalled differentiation resulting in a persistent embryonic state[97]. In paediatric tumours, such maturation blocks cause tumour cells to remain in a fetal-like state[96], but whether components of oncofetal reprogramming are also present, as they are in adult tumours, remains to be determined.

Paediatric tumours have clear ties to embryogenesis. Although all arise from cellular compartments that have not completed terminal differentiation, some differences are evident, on the basis of the underlying oncogenic events. For example, in acute lymphoblastic leukaemia, which is one of the most common childhood cancers, in utero genetic mutations predispose these patients to develop cancer[171]. However, cells bearing these pre-existing mutations might still require a second hit, such as a subsequent infection that alters their microenvironment, to become cancerous[172]. This observation indicates that some childhood cancers start early in pregnancy, whereas others involve the acquisition of mutations during early childhood. Other childhood tumours arise from cellular populations that develop a maturation block resembling a fetal state, including astrocytoma and neuroblastoma[96]. Finally, some paediatric cancers, such as Ewing sarcoma and Hodgkin lymphoma, manifest themselves during puberty alongside hormonal changes. Whether oncofetal reprogramming could also play a role in the development of paediatric cancers remains to be investigated.

Although paediatric and adult cancers acquire oncogenic changes during different developmental stages (embryonic or neonatal versus adult), they also require or develop an immunosuppressive microenvironment that facilitates the growth of malignant cells, as indicated by the approval of immune checkpoint inhibitor therapy for subsets of patients with various paediatric cancers. Therefore, studies of oncofetal reprogramming of the tumour microenvironment that compare tumours arising in paediatric patients with those arising in adults (for example, hepatoblastoma versus HCC, or paediatric acute lymphoblastic leukaemia versus adult acute lymphoblastic leukaemia) are of interest.

Conclusions and future outlook

Oncofetal reprogramming of the tumour microenvironment is an emerging field, and its implications in homeostasis and regeneration remain to be fully investigated. For example, some organs (such as the liver) and some tissues (such as the endometrium, which goes through a monthly cycle of

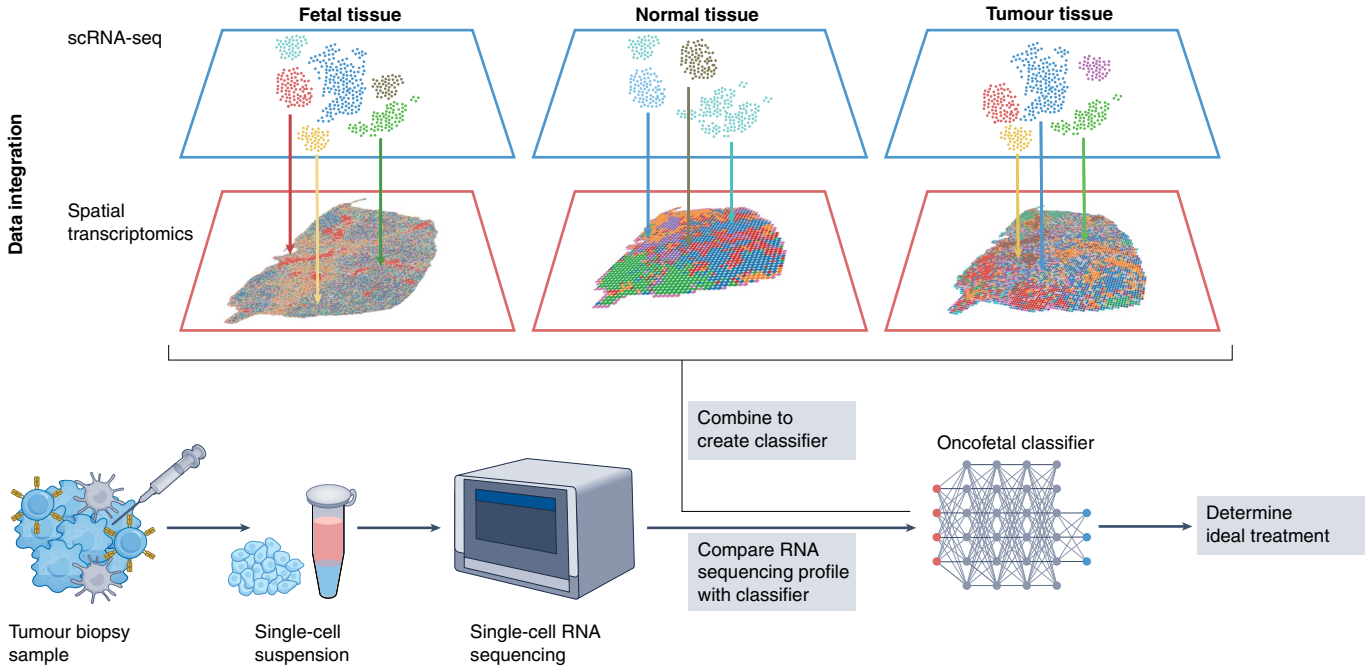

Fig. 4 | **Oncofetal score as a predictor of treatment response.** This schematic depicts the identification of oncofetal cells by comparative reference mapping of fetal, normal and tumour cell types. The addition of multimodal technologies, such as single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics, enables a comprehensive reference map of each state (fetal, normal and tumour) to be built. Machine learning could be used to develop an oncofetal ecosystem classifier that reveals the presence of oncofetal cells inpatient-derived tumour samples. Furthermore, identification of these oncofetal cells facilitates investigation of whether their presence can be used to predict the clinical outcomes of therapy, thereby enabling stratification of patients according to their probable response to therapy.

growth, differentiation and shedding) possess high regenerative capacity[173,174]. Therefore, oncofetal reprogramming might be an evolutionary trade-off in tissues with high regenerative potential. The likelihood of oncofetal reprogramming could also be useful in stratification of patients; for instance, in identifying which patients with cirrhosis or endometriosis (conditions associated with high levels of inflammation and/or regenerative potential) have an increased lifetime risk of developing liver or endometrial tumours. Such a stratification strategy might also help in the early detection and prevention of disease. Further studies are also needed to determine whether the phenomenon of oncofetal reprogramming is unique to cancer or might also play an important role in other inflammatory diseases, such as skin and neurological disorders[32,33].

In addition, the presence of oncofetal reprogramming could provide an opportunity to therapeutically target the tumour microenvironment. As an illustration, a combined immunotherapy for HCC (IMbrave150)[175–177], which comprises the anti-VEGF agent bevacizumab and the anti-PDL1 drug atezolizumab, both of which target components of the oncofetal tumour microenvironment[8], is emerging as a first-line therapy for HCC. We believe that such drug combinations could have an underestimated effect on oncofetal reprogramming and that the data from clinical trials of these agents could represent an unprecedented opportunity to validate this concept for therapeutic stratification. We hypothesize that the presence of oncofetal cells not only leads to an immunosuppressive microenvironment but also increases tumour cells' expression of molecules recognized by T cells, thereby making tumours 'hot' and increasing their susceptibility to immunotherapy (FIG. 4). However, these hypotheses remain to be tested in ongoing clinical trials of immunotherapy in HCC and other cancers. Therefore, oncofetal reprogramming of the tumour microenvironment appears as an emerging area of cancer research with implications ranging from tumour evolution to cancer immunotherapy.

Ankur Sharma[1,2,3,4]✉, *Camille Blériot*[5], *Jennifer Currenti*[1,2] *and Florent Ginhoux*[5,6,7,8]✉

[1]*Harry Perkins Institute of Medical Research, QEII Medical Centre, Nedlands, Western Australia, Australia.*

[2]*Curtin Medical School, Curtin University, Bentley, Western Australia, Australia.*

[3]*Curtin Health Innovation Research Institute, Curtin University, Bentley, Western Australia, Australia.*

[4]*Institute of Molecular and Cellular Biology, Agency for Science, Technology and Research (A*STAR), Singapore, Singapore.*

[5]*INSERM U1015, Institut Gustave Roussy, Villejuif, France.*

[6]*Singapore Immunology Network, Agency for Science, Technology and Research (A*STAR), Singapore, Singapore.*

[7]*Shanghai Institute of Immunology, Shanghai Jiao Tong University School of Medicine, Shanghai, China.*

[8]*Translational Immunology Institute, SingHealth Duke-NUS Academic Medical Centre, Singapore, Singapore.*

✉*e-mail: ankur.sharma@perkins.org.au; florent_ginhoux@immunol.a-star.edu.sg*

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