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Stem cell plasticity and tumour formation

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ABSTRACT

Stem cell plasticity refers to the ability of certain stem cells to switch lineage determination and generate unexpected cell types. This review applies largely to bone marrow cells (BMCs), which appear to contribute positively to the regeneration of several damaged non-haematopoietic tissues. This beneficial effect on regeneration may be a direct result of BMCs giving rise to organ parenchymal cells. Alternatively, it could be due to BMCs fusing with existing parenchymal cells, or providing paracrine growth factor support, or contributing to neovascularisation. In the context of oncology, BMC derivation of the tumour stroma and vasculature has profound biological and therapeutic implications, and there are several examples of carcinomas seemingly being derived from BMCs.

1. Introduction

Morbidity and mortality as a result of failing vital organs plagues even the most technologically advanced societies. Because of a dearth of transplantable organs there is a growing hope that stem cells may lead to the possibility of replacing tissues affected by age or disease. Indeed, it is almost impossible to open a newspaper today without seeing yet another apparent 'breakthrough' in stem cell research. Most adult tissues have *multipotential* stem cells; cells capable of producing a limited range of differentiated cell lineages appropriate to their location, e.g. small intestinal stem cells can produce all four indigenous lineages (Paneth, goblet, absorptive columnar and enteroendocrine), central nervous system (CNS) stem cells have trilineage potential generating neurones, oligodendrocytes and astrocytes,[1] whereas the recently discovered stem cells of the heart can give rise to cardiomyocytes, endothelial cells and smooth muscle.[2] However, describing tissue-based stem cells as 'multipotential' may be incorrect if, as it appears, some adult stem cells, when removed from their usual location can transdifferentiate into cells that arise from any of the three germ layers (so-called *plasticity*).

All tissues have stem cells, though in some tissues, notably brain and heart, they do not appear to be activated sufficiently adequately to replace damaged cells.

The resurgence of interest in stem cells has reaped dividends in terms of how we understand other diseases.

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Metaplastic and heterotopic changes from one recognisable tissue phenotype to another are well known in histopathology and are mostly seen in tissues with a high turnover of cells; such changes may result from genetic or epigenetic changes that affect expression of transcription factors, presumably in stem cells. For example, overexpression of the transcription factor Cdx2 targeted to the gastric epithelium, which does not normally express Cdx2, results in islands of intestinal metaplasia,[3] conversely the absence of Cdx2 expression in cdx2 null: wild type chimaeric mice results in patches of Cdx2 null gastric phenotype within wild type colonic mucosa[4]; importantly the junctional epithelium had the phenotype of small intestinal mucosa, despite being of wild-type heritage, and so their local stem cell units had adopted a specific relevant program of differentiation appropriate to their location.

Myofibroblasts are a distinguishing feature of pathological fibrosis, historically regarded as having originated by the activation of local parenchymal fibroblasts, and being the primary collagen-producing cells. However, such fundamental concepts will have to be reconsidered in the light of recent findings that bone marrow-derived cells contribute to fibrogenesis in both pulmonary[5] and hepatic scarring.[6] Moreover, bone marrow-derived cells are at least in part responsible for the tumour desmoplastic response[7] (see Fig. 6). Thus, bone marrow may provide a platform for the delivery of anticancer agents.

Since the classic 'initiation–promotion' experiments involving painting carcinogens on mouse skin,[8] it has been apparent that many cancers, particularly those of continually renewing tissues (blood, gut, skin), are in fact a disease of stem cells. These are the only cells that persist in the tissues for a sufficient length of time to acquire the requisite number of genetic changes for malignant development.[9] In fact, tumours are heterogeneous populations in which many cells are terminally differentiated (reproductively sterile) or transit amplifying cells with limited division potential, and so it seems that only tumour stem cells are capable of 'transferring the disease'. For example, in human acute myeloid leukaemia, only the $CD34^{+}$ $CD38^{-}$ cells are capable of propagating the disease in immunodeficient NOD/SCID mice,[10] while in human breast cancer, the $CD44^{+}$ $ESA^{+}CD24^{-/low}$ fraction has a similar potential.[11] In the central nervous system (CNS), CD133 appears to be expressed on those cells with the greatest clonogenic potential in vitro,[12] and these CD133-positive cells are the ones that give rise to further medulloblastomas in NOD/SCID mice.[13] Therefore, there is a growing conviction that successful cancer chemotherapy depends upon eradicating all the stem cells within a cancer. This review focuses on bone marrow stem cell plasticity, how bone marrow could be the origin of some carcinomas, and how other cells of bone marrow origin, notably myofibroblasts and endothelial progenitor cells (EPCs) could be exploited therapeutically.

Stem cells feature prominently in disease processes; metaplasia illustrates stem cell plasticity, the bone marrow is a source of fibrogenic and endothelial progenitor cells, normal stem cells are the likely carcinogen targets, and cancers themselves probably all have stem cells.

2. Adult stem cell plasticity

A large body of evidence now supports the idea that certain adult stem cells, particularly those of bone marrow origin, can engraft alternative locations (e.g. non-haematopoietic organs), particularly when the recipient organ is damaged, and transdifferentiate into cell types with functions appropriate to their new location (Fig. 1). Hence, there is considerable excitement in exploring the use of haematopoietic stem cells (HSCs) in cell-based therapies and as vectors to deliver therapeutic genes. This is particularly attractive to the clinician because bone marrow stem cells can readily be obtained from patients simply by mobilising HSCs into the peripheral circulation by injection of a cytokine such as G-CSF. Moreover, if a patient's own stem cells were taken for ex vivo expansion and directed to differentiate into say, liver cells or brain cells, no immune rejection problems would arise.

However, it is worth noting that not everyone is convinced of the versatility of stem cells (reviewed in[14]). Doubts have been raised because certain instances of the so-called plasticity have now been attributed to cell fusion between bone marrow cells (or their macrophage descendants) and cells of the recipient organ. Furthermore, several remarkable claims have not been confirmed in other laboratories. Lastly, while a scattering of engrafted cells of haematopoietic origin (but with a phenotype appropriate to their new location) is often observed in damaged parenchymal organs, these cells appear to have engrafted not as stem cells but either as transit amplifying or terminally differentiated cells, thus their long-term value is questionable. Fig. 2 summarises how BMCs may influence organ healing, and it is becoming clear that, as well as a direct involvement through plasticity, bone marrow-derived cells can have an influence through promoting vasculogenesis and as a source of paracrine-acting growth factors. Of course, these indirect effects could have profound effects on tumour growth.

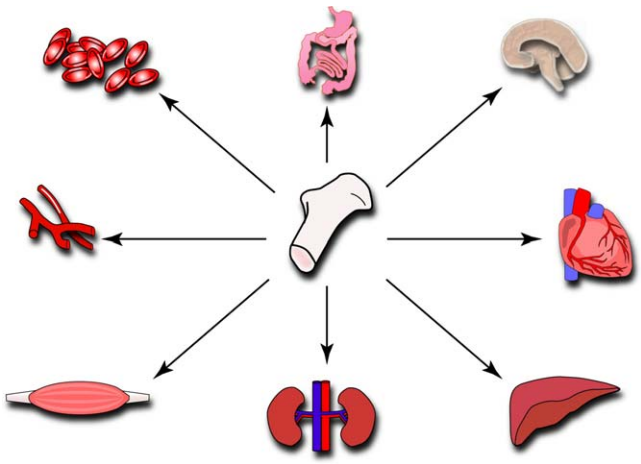

Fig. 1 – Adult stem cells, particularly those from the bone marrow, may under certain circumstances migrate to damaged organs, engraft and transdifferentiate into cells of that organ.

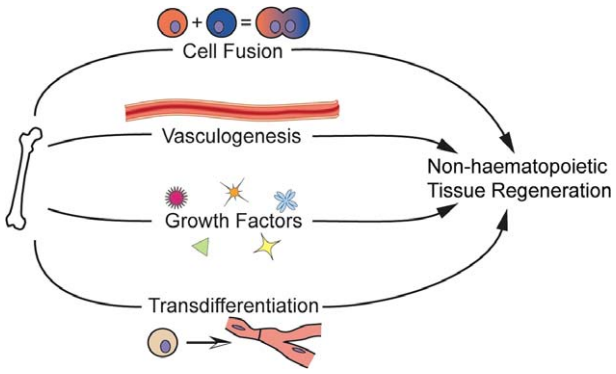

Fig. 2 – There are at least four mechanisms through which bone marrow cells (BMCs) might aid non-haematopoietic organ regeneration (see text for details).

2.1. *Cell fusion*

Claims for adult stem cell plasticity often rely on the appearance of Y chromosome-positive cells in a female recipient of a male bone marrow transplant. Alternatively, markers such as LacZ or green fluorescent protein (GFP) have been used, and these techniques are usually combined with lineage markers in attempts to demonstrate there has been a switch in the fate (transdifferentiation) of the transplanted cells (Fig. 3). Numerous papers claim that adult BMCs can differentiate into all manner of tissues, including skeletal muscle, cardiomyocytes and endothelia, neurones and glia, hepatocytes and bile duct epithelia, renal epithelia and podocytes, and gut mucosal cells and associated myofibroblasts (reviewed in[14]). Since most observations have been made in cases of sex-mismatched bone marrow transplantation, the obvious step regarding cell fusion is to examine the cells for the presence of X and Y chromosomes: if cell fusion was responsible

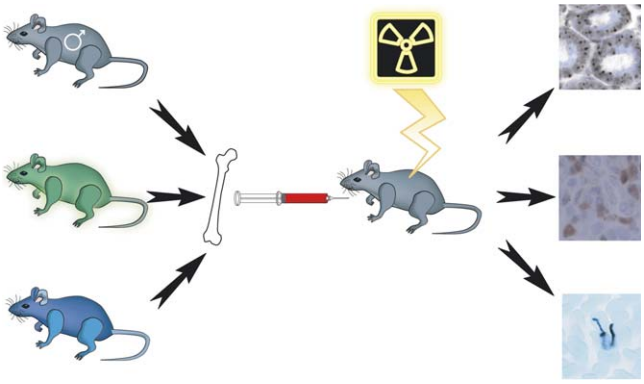

Fig. 3 – Revealing that bone marrow cells (BMCs) have differentiated into non-haematopoietic cells can be achieved by transplanting lethally irradiated animals with new BMCs that can be tracked whatever their subsequent fate. This would include male BMCs to a female recipient, or green fluorescent protein (GFP)- or LacZ-positive BMCs to wild-type recipients. The male chromosome can be detected by in situ hybridisation, GFP by immunohistochemistry and β-galactosidase by X-gal histochemistry.

then the donor (apparently) transdifferentiated cell would have an XXXY karyotype rather than XY of a purely transdifferentiated male donor, and would therefore be a heterokaryon. The major drawback to this type of analysis is that most are performed on paraffin wax-embedded tissue sections of finite thickness – even in routine 4–6 μm thick sections of male control tissue, the Y chromosome (located usually at the nuclear periphery) is only detected in 50–60% of male cells, thus, the likelihood of 'missing' the extra chromosomes present in a fusion cell is high. This was not a problem encountered by Tran and colleagues,[15] who analysed almost 10,000 thin buccal cells from five female recipients of CD34+ BMC transplants from male donors. Most significantly, they could detect 98% of Y chromosomes in male control cells and found two X chromosomes in 99% of female control cells. The number of Y- and cytokeratin 13-doubly positive buccal cells in the female recipients ranged from 0.8% to 12.7%, with only one XXXY cell (0.01%) and one XXY cell (0.01%) detected, both of which could have arisen by fusion. Not unreasonably, they concluded that BMCs could transdifferentiate directly into buccal cells without cell fusion.

Diabetes is a major cause of morbidity and mortality, but islet cell transplantation is a treatment only available for a tiny minority of sufferers. Thus, the possibility that BMCs could be used to generate β-cells is very attractive. Adopting a very elegant genetic approach, male mouse BMCs transplanted into lethally irradiated female recipients were found to transdifferentiate, again, without fusion, into pancreatic islet cells possessing many markers of β-cell differentiation and the ability to secrete insulin in response to glucose.[16] However, in undoubtedly the most convincing 'proof of principle' demonstration of the potential therapeutic utility of bone marrow, mice with a metabolic liver disease have been cured.[17] Female mice deficient in the enzyme fumarylacetoacetate hydrolase (*Fah*−/−, a model of fatal hereditary tyrosinaemia type 1), a key component of the tyrosine catabolic pathway, can be rescued biochemically by a million unfractionated BMCs that have the Fah enzyme. The salient point from this demonstration of the therapeutic potential of BMCs was that, although the initial engraftment was low, approximately one BMC for every million indigenous hepatocytes, the strong selection pressure exerted by liver failure on the engrafted BMCs resulted in their clonal expansion eventually to occupy almost half the liver. However, the new 'healthy' liver cells in the engrafted *Fah*−/− mouse contain chromosomes from both the recipient and donor cells, with the donor haematopoietic cell nuclei being reprogrammed when they fused with the unhealthy *Fah*−/− hepatocyte nuclei to become functional hepatocytes.[18] Interestingly, many of these 'rescued' hepatocytes had an aneuploid karyotype, which could have implications for hepatocarcinogenesis. The fusion partners in the Fah setting are not the HSCs themselves, but are bone marrow-derived macrophages (BMMs) and granulocyte/macrophage progenitors (GMPs).[19,20] In one study, T and B cells were found not to be involved, and direct intrasplenic transplantation of either BMMs or GMPs, without lethal irradiation or haematopoietic reconstitution, led to robust replacement of the Fah null liver with Fah^{+} hepatocytes.[19] Likewise, a similar conclusion can be drawn from a series of experiments including a Cre/lox strategy, in which bone

marrow was transplanted from mice that expressed Cre recombinase only in the myelomonocytic lineage to *Fah*−/− mice that had a floxed β-galactosidase reporter gene; nodules that were positive for both β-galactosidase and Fah indicated that probably Kupffer cells were the fusion partners for the Fah-deficient hepatocytes.[20]

Fusion of BMCs has also been found to occur in the normal mouse, not only with hepatocytes, but also with Purkinje cells in the brain and cardiomyocytes.[21] These were very elegant studies both in vivo and in vitro, in which a reporter gene was activated only when cells fused. However, unlike the *Fah*−/− mouse, no selection pressure (liver damage) was operative, even after 10 months only 9–59 fused cells/5.5×10^5 hepatocytes were found: importantly, they also found evidence that, with time, either certain donor genes had been inactivated or eliminated, suggesting genetic instability in such heterokaryons. The significance of these examples of aneuploidy for tumour development remains to be ascertained. On the other hand, other data suggest that under normal physiological circumstances true transdifferentiation rather than cell fusion prevails.[22] In this study, lethally irradiated female mice that ubiquitously expressed Cre recombinase were the recipients of male bone marrow that would only express enhanced green fluorescent protein (EGFP) if fusion occurred; 2–3 months after transplantation 0.05% of 36,000 hepatocytes were Y chromosome positive, but none expressed EGFP.

On the issue of cell fusion, it would seem reasonable to conclude that, apart from in the liver, heart and Purkinje cells (also skeletal muscle, where fusion is the mechanism by which myotubes are generated), there is little evidence for cell fusion between bone marrow-derived cells and parenchymal cells. However, because such a mechanism can occur, it is now mandatory that all investigations at least consider the possibility, notwithstanding the fact that there is nothing inherently wrong in correcting a metabolic deficiency by cell fusion. There is more direct evidence for lineage switching; cultured pancreatic cell lines can readily differentiate in vitro into hepatocytes,[23] moreover, the induced transdifferentiation commonly occurred directly without cell cycle traverse and involved the vast majority of a pure population of exocrine pancreatic cells – an occurrence that could not involve cell fusion with another cell type. Likewise, when murine HSCs were co-cultured with injured hepatocytes, but separated by a trans-well barrier to prohibit cell fusion, differentiation of many of the HSCs to cells with an hepatocyte-like phenotype occurred within 2 d.[24]

Rather than direct transdifferentiation, cell fusion can occur between HSCs and parenchymal cells, reprogramming the HSC genome; nevertheless a clear sign of the plasticity of the HSC phenotype.

2.2. *Reproducibility*

A major issue that has exercised both protagonists and antagonists of adult stem cell plasticity has focused on the reproducibility of certain remarkable claims. For example, Bjornson and colleagues[25] demonstrated that single LacZ+ neural stem cells could form large colonies (neurospheres) in vitro that had all three neural lineages present, and that such neurosphere cells also had haematopoietic potential when transplanted into sub-lethally irradiated mice. An in vitro clonogenic assay of the bone marrow from the transplanted mice showed that most (~95%) of the colonies were positive for β-galactosidase suggesting that they were of neural stem cell origin. Significantly, cultured neural stem cells neither proliferated nor formed haematopoietic progeny in the same clonogenic assays without prior injection into irradiated host mice, indicating that an appropriate microenvironment was necessary for transdifferentiation. However, another study using a similar protocol rigorously tested the haematopoietic potential of murine neurosphere cells and was unable to find any evidence whatsoever of haematopoietic differentiation in a group of 128 sub-lethally irradiated mice each transplanted with 1×10^6 neurosphere cells, which suggested that a haematopoietic potential was not a general property of neural stem cells.[26] The therapeutic potential of bone marrow for the treatment of liver disease is also fiercely debated, with some animal models claiming significant and sustained ingress of BMCs that become hepatocytes. However, most long-term studies of human liver allografts fail to find any significant chimaerism in the graft, despite one report claiming 40% of hepatocytes were derived from the recipient (reviewed in[27]).

Perhaps the most contentious area in this field concerns the myocardium; a murine model of infarction was used to claim that direct injection of Lin^- c-kit^+ HSCs into the peri-infarct zone results in their rapid (within 9 d) transdifferentiation to cardiomyocytes.[28] However, no such conversion was noted by other groups using similar models,[29,30] a failure attributed by the former group to technical error.[31] Nevertheless, a number of medical centres worldwide, including some in the United Kingdom (UK), are claiming a significant clinical benefit results from the injection of autologous bone marrow to patients with either congestive heart failure[32] or who are recovering from myocardial infarction.[33] Bone marrow is, of course, a well-recognised source of EPCs, and these EPCs may well account for much of the benefits of BMC infusion in heart disease patients (Fig. 2). In the pancreas, the role of BMCs in β-cell renewal is unclear; one study claimed that up to 3% of β-cells could be bone marrow derived shortly after a bone marrow transplant to mice with no obvious pancreatic damage,[16] whereas another study adopting a familiar model of β-cell damage, though noting a beneficial affect of bone marrow transplantation, attributed this to an improved islet blood vasculature.[34]

Conflicting observations have also been made regarding the ability of bone marrow to contribute to neural tissue. For example, homozygous PU.1 mutant female mice (PU.1 is a transcription factor required for the histogenesis of six of the haematopoietic lineages) were rescued with a bone marrow transplant from male wild-type donors, latterly it was found that up to 4.6% of cells in the CNS were Y chromosome-positive, and that up to 2.3% of Y-positive cells possessed the neuronal markers NeuN and neuron-specific enolase (NSE).[35] The same laboratory has found small numbers of Y chromosome-positive cells bearing neural antigens in post-mortem tissues of female patients who had had a bone marrow transplant from a male donor a

few months before.[36] On the other hand, no neuronal differentiation was found in eight lethally irradiated recipients of 2×10^3 SP cells from ROSA26 donors nor in 12 recipients of 2×10^6 whole BMCs, even though some of the recipients in both groups had a neuronal injury.[37] What are we to make of these last discrepancies? Mezey and colleagues considered that perhaps engrafted cells were missed through technical faults in detecting the protein products of transgenes such as LacZ and GFP, compounded by the fact that it is virtually impossible to get ubiquitous transgene expression in all tissues.[38] We would also add that in each case the experimental conditions were not identical. Recent data has also been very contrasting; Massengale and colleagues[39] examined more than 10,000 cells of bone marrow origin in bone marrow-transplanted animals with healthy and injured brains and found no evidence that these cells differentiated into neural cells, concluding BMCs maintain lineage fidelity in the brain. On the other hand, Bonila and colleagues[40] observed not only transdifferentiation of $CD117^+$ HSCs into neural cell types after their transplantation into neonatal mouse brains, but also their ability to form self-renewing neural stem cells.

In the kidney, the mechanisms by which bone marrow-derived cells provide protection against acute renal failure (ARF) can be seemingly quite different.[41,42] For example, Morigi and colleagues[43] found that injected mesenchymal stem cells (MSCs) were renoprotective after cisplatin-induced ARF in mice, and that these cells also engrafted and acquired a tubular phenotype. However, Togel and colleagues[44] noted no such engraftment of MSCs after ischaemia-induced ARF in rats, concluding that the marked beneficial effects of MSCs were most likely to accrue from paracrine effects, perhaps by modulating T cell activity. On the other hand, Fang and colleagues[45] observed not only renal engraftment of BMCs after folic acid-induced tubular injury, but also their ability to undergo DNA synthesis as part of the regenerative response.

Controversy also surrounds the claim that just a single cell from male mouse bone marrow (lineage-depleted and enriched for $CD34^+$ and $Sca\text{-}1^+$ by in vivo homing to the bone marrow), can, when injected into female recipients along with 2×10^4 female supportive haematopoietic progenitor cells, give rise to a spectrum of epithelial cells: at 11 months a surprisingly high proportion of type II pneumocytes were Y chromosome-positive, though fewer Y chromosome-positive cells were seen in other tissues, e.g. 2% were cytokeratin-positive in the skin.[46] The high level of lung engraftment was attributed to lung damage caused either by the irradiation to eradicate endogenous bone marrow to facilitate bone marrow transplantation or by viral infection in the temporarily immunocompromised animals. Though the experiments are not directly comparable, the observations of Wagers and colleagues[47] led the authors to speculate that 'transdifferentiation of circulating HSCs is an extremely rare event if it occurs at all'! In one approach, they transplanted GFP-marked HSCs into lethally irradiated non-transgenic recipients, and although GFP^+ HSCs colonised the bone marrow, no significant contribution was made by these cells to epithelia. The other approach involved the long-term study of parabiotic pairs between GFP^+ mice and wild-type mice, and once again significant chimaerism was observed in the bone marrow, but not in other organs.

The derivation of cardiomyocytes from HSCs is controversial in murine models, but the direct intracardiac injection of autologous HSCs appears to benefit patients with cardiovascular disease, perhaps through aiding neovascularisation.

3. Patterns of bone marrow cell engraftment

Apart from the murine Fah knock-out liver failure model,[17–20] a notable feature of most studies reporting plasticity is that BMCs do not engraft as stem cells or at least cells with any degree of clonal expansion capability. If they did, then one would expect to see patches of bone marrow-derived cells, especially in renewing tissues such as the gut epithelium and epidermis. This has not been the experience in the gut,[48–50] nor generally in epidermis,[51] though a recent study has found evidence of BMCs giving rise to all the cells in a 'epidermal proliferative unit', a structure considered to be dependent on the descendants of individual stem cells.[52] If BMCs did engraft as stem cells in a new location, it is not inconceivable that they could be the founder cells of tumours at these locations. Indeed, such an association has been claimed for *Helicobacter*-related murine gastric cancer, where ablation of the indigenous stem cell compartment because of protracted tissue damage (gastritis), has resulted in gastric adenocarcinoma being derived from BMCs,[53,54] possibly MSCs

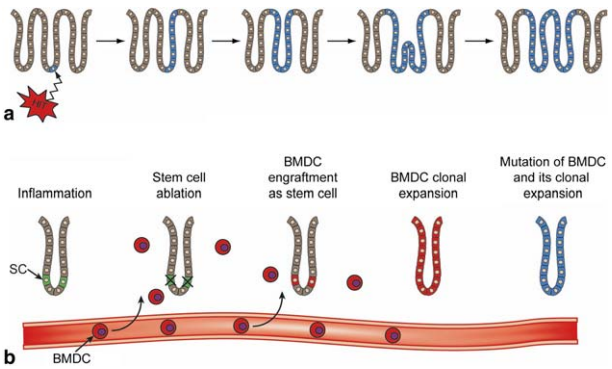

Fig. 4 – (a) A conventional model for the development of early colorectal cancer (CRC). Mutation occurs in a stem cell located near the base of the crypt and mutated cell progeny occupy part of the crypt. Through a stochastic process (called 'niche succession' or 'monoclonal conversion') the affected crypt becomes wholly occupied by dysplastic cells – the monocryptal adenoma. Further expansion can occur by the dysplastic crypt undergoing crypt fission and budding, leading to an oligocryptal adenoma (aberrant crypt focus). Key: normal colonocytes are brown, mutated colonocytes are blue. (b) A new paradigm of epithelial cancer development. Continued tissue damage leads to loss of the indigenous stem cell compartment and its replacement by bone marrow derived cells (BMDCs), whose progeny subsequently repopulate the whole crypt. Mutation in a BMDC engrafted as a stem cell can then lead to adenoma formation as described in 4a. Key: indigenous normal epithelial cells are brown, indigenous stem cells are green, BMDCs are red and mutated BMDCs are blue.

rather than HSCs (Fig. 4). An origin of carcinoma from BMCs has also been suggested for one case of skin basal cell carcinoma (BCC) arising in a female recipient of a male kidney transplant.[55] In this case, most of the cytokeratin-positive tumour cells were male, and since BCC rarely, if ever, metastasises (so no occult metastasis in the transplanted organ), it is likely that donor BMCs in the graft had migrated to the skin, either fusing with or differentiating into keratinocytes, before undergoing malignant transformation.

Cell fusion or nuclear fusion, also called synkaryon formation,[56] as described in the tyrosinaemic mouse,[18] may also have implications for tumourigenesis. Such a process could endow differentiated cells with stem cell properties such as infinite self-renewal, while at the same time result in genetic instability with obvious tumourigenic potential.

4. Alternative roles of BMCs in tumour development

The bone marrow may indirectly influence tumour behaviour through a contribution to the desmoplastic response and to the tumour vasculature (Fig. 5). EPCs constitute a unique population of peripheral blood mononuclear cells derived from bone marrow that are involved in postnatal angiogenesis during wound healing, limb ischaemia, post-myocardial infarction, atherosclerosis and tumour vascularisation. HSCs and EPCs are seemingly derived from a common precursor, called a hemangioblast, so in the bone marrow these cells share many antigenic determinants, including CD34, CD133, Sca-1, c-Kit, Tie-2 and Flk-1.[57–59] However, once in the circulation, these cells express markers of endothelial commitment, including von Willebrand factor (vWF) and VE-cadherin.[58] The primitive EPC is probably best defined as $CD133^+$ $CD34^+$ $Flk\text{-}1^+$. Circulating EPCs are mobilised endogenously in response to tissue ischaemia or exogenously by cytokine therapy to augment neovascularisation. Davidoff and colleagues[60] convincingly demonstrated the therapeutic potential of BMCs by transducing them with a retroviral vector encoding a soluble, truncated form of VEGF-2 (Flk-1; tsFlk-1); when transplanted into mice bearing neuroblastomas they were found to engraft into the neovasculature and very significantly impede tumour growth. In transgenic mice engineered to develop mammary tumours, Dwenger and colleagues[61] observed that BMCs preferentially home to the blood vessels of the tumour. In humans also, BMCs contribute to tumour vasculature: Peters and colleagues[62] observed that up to 12% of endothelial cells can be from the bone marrow. All these observations indicate BMCs can be important delivery agents for anti-tumour gene therapies as well as for conventional anti-angiogenic drugs.

Myofibroblasts are cells with features of both smooth muscle cells and fibroblasts. They are widely distributed, having roles in growth and differentiation as well as in the inflammatory response. They are also important in injury and contribute to the processes of fibrosis and scarring, where they produce extracellular matrix proteins such as the interstitial collagens (reviewed in[63]). We and others have shown that the bone marrow contributes to myofibroblast populations throughout the body.[64] This bone marrow contribution is up-regulated by injury such as skin wounding,[64,65] radiation-induced lung injury,[66] experimental colitis[67] and in human liver damage.[6] We have also shown that the bone marrow contributes to myofibroblast populations in the human gastrointestinal tract.[68] Bone marrow also contributes to myofibroblast populations in tumour stroma in mouse models.[7,69,70] We have shown this in murine pancreatic insulinoma (Fig. 6), while Ishii and colleagues have made similar observations in mice xenotransplanted with various human cancer cell lines.[69,70]

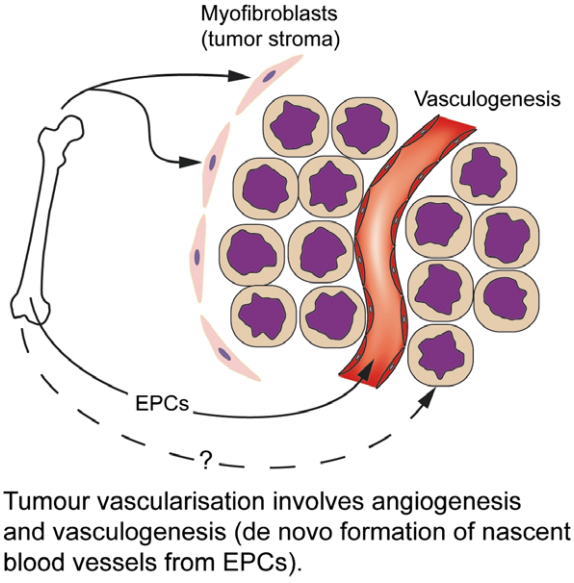

Fig. 5 – In the context of tumours, the myofibroblasts and fibroblasts comprising the tumour stroma can be derived from the bone marrow, as can many of the endothelial cells of the neovasculature (vasculogenesis). Gastric carcinomas in mice have been found to arise from bone marrow cells (BMCs) engrafted in the gastric epithelium. Whether many human carcinomas have a similar origin is unknown, but it will be difficult to ascertain.

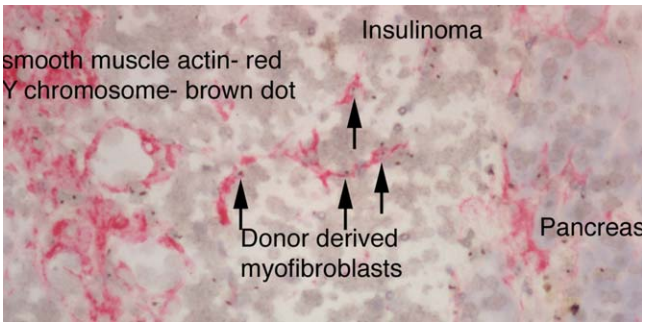

Fig. 6 – Mouse model of pancreatic insulinoma demonstrating the contribution of bone marrow to the desmoplastic reaction. This female mouse had a bone marrow transplant from a male donor 2 months beforehand. The tumour (left-hand side) is surrounded by myofibroblasts that express α-smooth muscle actin (α-SMA) (red stain), and also have a Y chromosome (brown dot) indicating their origin from the bone marrow transplant.

It is highly likely that these myofibroblasts originate from MSCs, cells that act as a source of progenitors for a variety of mesenchymal tissues.[71] Abe and colleagues[72] found peripheral blood circulating *fibrocytes* that express collagen type I, which rapidly entered sites of injury. When cultured, these cells had the ability to contract collagen gels and express α-smooth muscle actin (α-SMA); such fibrocytes are also likely to be derived from MSCs.

The significance of such observations is that it is becoming increasingly evident that tumour-associated myofibroblasts and fibroblasts have a very active role in tumourigenesis. For example, in hepatocellular carcinoma and hepatoblastoma, hepatocyte growth factor (HGF) produced by tumour-associated myofibroblasts undoubtedly promotes invasion and tumour growth,[73,74] while in many other tumour types, growth factors such as transforming growth factor (TGF)β and insulin-like growth factor (IGF) are produced (reviewed in[63]). Ohuchida and colleagues combined a pancreatic cancer cell line with irradiated or non-irradiated fibroblasts prior to transplant into recipient mice, and the irradiated fibroblasts allowed a more aggressive cancer phenotype.[75] Tumour encapsulation has been shown to be associated with improved survival in hepatocellular carcinoma,[76] while the abundance of reactive stroma is an independent marker of prostate cancer progression,[77] and tumour aggressiveness in urothelial carcinoma.[78] The latter two observations highlight the active role of the stroma in tumour progression, indeed in colorectal and breast carcinomas the stromal cells have been found to be a rich source of the basement membrane degrading enzyme gelatinase A, also called matrix metalloproteinase 2 (MMP-2).[79,80]

These observations have paved the way for utilising MSCs in cell and gene therapy. For example, MSCs have been transduced with an adenoviral vector carrying the human interferon β gene and injected into immunodeficient mice with established xenografted human tumours, resulting in a significant improvement in survival compared with controls.[81,82] The stromal myofibroblast is certainly a key player in the control of tumour cell behaviour[83] and will surely be exploited in the development of many new anticancer strategies.

Though bone marrow plasticity may result in carcinomas being derived from BMCs, this is difficult to verify in humans. However, the bone marrow commonly exerts an influence on cancer growth through its contribution to tumour neovasculature and the tumour stroma. This bone marrow to tumour axis is already being exploited for tumour therapy in pre-clinical models.

5. Conclusion

In terms of therapeutic potential for regenerative medicine, apart from embryonic stem cells and adult BMCs, there are other sources of related stem cells that might be amenable to manipulation. These would include cord blood, where cells with a wide differentiation repertoire named 'unrestricted somatic stem cells' (USSC) have recently been described,[84] and even the matrix supporting the umbilical cord; the so-called Wharton's jelly.[85] There are also the 'multipotent adult progenitor cells' (MAPCs) isolated from mouse, rat and human MSC cultures, cells that appear capable of differentiating into most, if not all somatic cell types.[86] On the other hand, others have suggested that bone marrow plasticity is nothing more than a reflection of the bone marrow being a heterogeneous population containing, in addition to HSCs, MSCs and EPCs, the so-called tissue-committed stem cells (TCSCs), cells already committed to non-haematopoietic lineages.[87,88] These very rare cells in human bone marrow express CD34, CD133 and the chemokine receptor CXCR4, but are lineage-negative and importantly, *do not* express the pan-haematopoietic marker CD45. It is envisaged that these TCSCs 'hide-out' in the bone marrow, being recruited to damaged organs due to the up-regulation of chemokines such as SDF-1, and it is further suggested that bone marrow-located TCSCs become scarcer with age, contributing to the poorer wound healing qualities of older tissues.

There are other sources of malleable stem cells; even liposuction waste has 50–100 million stem cells per 250 g, useful for the generation of fat, bone and cartilage. Adipose-derived adult stem cells (ADAS cells) have been described as $CD31^-$, $CD34^-$, $CD160^-$ ($VCAM\text{-}1^-$) and $Flk1^+$, and found to be very effective EPCs.[89] The dermis may also be a source of unusually versatile stem cells; the so-called skin-derived precursors (SKPs) have been isolated from the dermal papillae of hair follicles.[90]

Epithelial-mesenchymal transitions (EMTs) are a very common occurrence during embryonic development, as is the reverse process (MET); clear examples of cell phenotype plasticity (Fig. 7). Moreover, EMT also occurs in chronic fibrotic disease, e.g. in renal interstitial fibrosis, largely mediated by the actions of TGF-β1. EMT is often described in the late

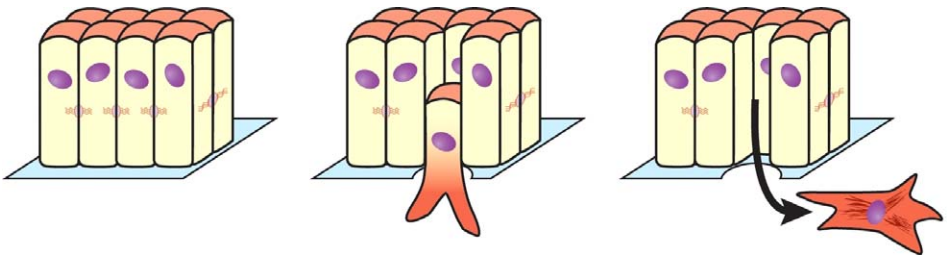

Fig. 7 – Epithelial-mesenchymal transition (EMT) involves loss of cell–cell adhesion, detachment from underlying connective tissue, induction of motility and acquisition of the mesenchymal phenotype, e.g. expression of α-smooth muscle actin (α-SMA) and synthesis of collagens. Carcinoma cells may acquire such a mesenchymal phenotype that facilitates an invasive habitus.

stages of tumour progression, allowing epithelial cells to become motile, eventually metastatic cells.[91,92] Such tumours, the so-called 'metaplastic carcinomas', typically express mesenchymal markers, such as matrix metalloproteinases.[93] However, not everybody is convinced of the need to invoke a change in cell identity to explain neoplastic cell behaviour, believing EMT to be a fallacy, and that all such cell alterations can be encompassed within the neoplastic cell's normal repertoire.[94] Finally, in this review of plasticity and its relevance to neoplasia, we should note a recent remarkable claim that the bone marrow can repopulate the sterilised mouse ovary with oocytes, suggesting that BMCs could be used to restore fertility in female chemotherapy patients.[95]

Embryonic stem cells are not the only source of versatile (plastic) stem cells, they can be isolated from bone marrow, peripheral blood, cord blood and Wharton's jelly, liposuction waste and even dermal tissue. Many carcinomas adopt a more mesenchymal phenotype as they progress (a type of plasticity), facilitating invasion and metastasis.

Conflict of interest statement

None declared.

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