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SPECIAL ARTICLE

MACROPHAGES, MORE THAN JUST SCAVENGERS: THEIR ROLE IN BREAST DEVELOPMENT AND CANCER

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Macrophages, derived from circulating monocytes, are located within virtually all adult tissues. There, these specialized cells adopt tissue-specific functions that are important for normal tissue homeostasis and response to physiological challenges. Increasing evidence suggests that macrophages play a role in the normal development of certain organs, such as the breast. Intriguingly, macrophages are often found in the stroma of breast tumours, where they may promote tumour growth and metastasis. In this review we discuss this emerging area of developmental and tumour biology.

Key words: breast cancer, colony stimulating factor-1, mammary stem cell, metastasis, stem cell niche, tumour-associated macrophage, vascular endothelial growth factor.

Abbreviations: IL, interleukin; MCP-1, monocyte chemotactic protein-1; M-CSF-1, macrophage colony-stimulating factor-1; MSP, macrophage-stimulating protein; TAM, tumour-associated macrophage; TEB, terminal end bud; TGF, transforming growth factor; TNF, tumour necrosis factor; VEGF-A, vascular endothelial growth factor-A.

INTRODUCTION

Breast development requires a complex interaction between the stroma and epithelium that ultimately form the mature ductal and lobular structures found in the adult breast. The stroma also plays a regulatory role in controlling epithelial differentiation during pregnancy and lactation.[1] Similarly, tumour cells show some dependence on interactions with surrounding stromal tissue.[2] Fibroblasts and adipocytes in the stroma of the pubertal mammary gland have long been known to regulate the branching and growth of the mammary epithelial tree.[3] The role of other stromal components, such as macrophages and other inflammatory cells has only recently begun to be appreciated.

MACROPHAGE BIOLOGY

Circulating monocytes are derived from a common myeloid progenitor cell. These cells migrate to various organs and undergo differentiation into specialized resident macrophages, where they are usually designated by their organ of residence, for example, Kupffer cells in the liver, microglia in the brain, osteoclasts in the bone and mesangial cells in the kidney. The observation of macrophages in mature organs has been aided by a *fms-EGFP* transgenic mouse in which macrophages are recognized by their green fluorescence.[4]

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The diversity of macrophage functions has led to their further subclassification based on their cellular behaviour and response to cytokines. 'Classically' activated (M1) macrophages are involved in phagocytosis and production of pro-inflammatory cytokines. They are induced by γ-interferon, often in concert with microbial stimuli (through surface lipopolysaccharides), or tumour necrosis factor (TNF)-α. 'Alternatively' activated (M2) macrophages are a heterogeneous group of cells induced by the T-helper 2 cytokines interleukin (IL)-4 and IL-13, as well as glucocorticoids and transforming growth factor (TGF)-β.[5] They have a role in tissue remodelling and repair and also carry out immunoregulatory functions and promote angiogenesis. M2 macrophages can execute some or all these tasks during tumour development and metastasis.

MACROPHAGES AND MAMMARY GLAND DEVELOPMENT

The key physiological features of human breast development are recapitulated in mouse models. The mouse mammary gland develops in distinct phases that include embryogenesis, puberty, pregnancy, lactation and involution. At birth, the mouse mammary gland is a small rudimentary structure that remains relatively dormant until puberty. At that time, in response to hormones (including oestrogen, progesterone and prolactin), rapid ductal elongation and branching of epithelium from the nipple region occurs, eventually resulting in complete filling of the mouse mammary fat pad (Fig. 1a). At the growing end of the ducts are specialized structures, called terminal end buds (TEB), which coordinate the ductal outgrowth. During pregnancy, further ductal side branching occurs, together with the appearance of lobuloalveolar structures that undergo functional differentiation as milk-secretory units required for lactation. This phase is regulated by progesterone and pregnancy-induced hormones. After

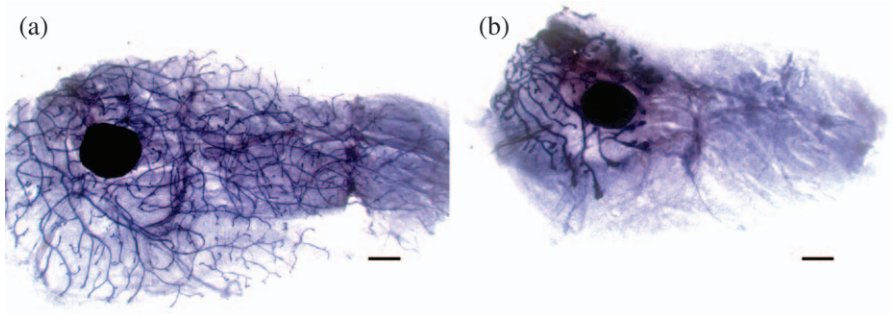

Fig. 1. Mammary gland development is perturbed in macrophage-deficient mice. (a) Mammary gland wholemount (fourth) from a wild-type female mouse at 8 weeks of age showing complete filling of the fat pad with complex branching ductal structures. Terminal end buds have disappeared by this stage. (b) Age-matched *op/op* mammary gland shows incomplete filling of the mammary fat pad, fewer ductal branches and persistence of terminal end buds. The central structure is a normal intramammary lymph node. Bar = 1 mm.

weaning, regression of lobuloalveolar structures occurs through apoptosis and remodelling of both the epithelium and stroma.

Macrophages have been implicated in these developmental processes: both macrophages and eosinophils are recruited to the growing TEB and are found around the neck and head of the TEB, respectively.[6] Macrophage colony-stimulating factor 1 (M-CSF-1) is a key growth factor controlling the proliferation and survival of the mononuclear phagocyte lineage. Extensive investigation of macrophage biology has been carried out in '*op/op'* mice, which harbour a mutation in the *CSF-1* gene.[7–9] *Csf1 op/op* mice have reduced macrophage numbers in most tissues, including the mammary gland. They are runted, toothless and osteopetrotic because of osteoclast deficiency impairing bone resorption. *Op/op* mammary glands show restricted TEB outgrowth and ductal branching (Fig. 1b).[10] Studies in these mice have shown that the absence of macrophages results in defective collagen deposition which partly explains the disorganized terminal end buds.[11] During pregnancy, *op/op* mice have a lactational defect and develop precocious lobuloalveolar outgrowths, but fail to switch to the lactational state.[12]

To confirm that the observed mammary phenotype was due to the local deficiency of macrophages (rather than to systemic or endocrine deficiency), a transgenic mouse was developed that specifically restored *Csf-1* expression in the mammary gland.[13] While still manifesting the other abnormalities of the *op/op* mice, these mice had normal mammary epithelial architecture with restoration of resident mammary macrophages. Thus macrophages are required for normal branching morphogenesis in the mammary gland.

A ROLE IN THE STEM CELL NICHE?

The stem cell niche is formed by a specialized group of cells and extracellular matrix that provide an environment to sustain the stem cell, thereby enabling key stem cell properties of self-renewal and pluripotency.[14,15] In the colonic epithelium, macrophages are located in the pericryptal stem cell niche.[16] The stem cells are located at the base of the colonic crypt and with differentiation its progeny migrate up the crypt towards the lumen. In response to colonic injury, macrophages have been shown to not only express genes associated with stem cell activation but also extend processes to make direct contact with the colonic epithelial progenitor cells.[16] The macrophages appear to be the vehicles by which the inflammatory signals of injury are transmitted to activate the stem cell. Similarly, osteoclasts have been shown to play a role in haematopoiesis by regulation and mobilization of haematopoietic stem cells in the endosteal stem cell niche.[17] It is possible that the macrophages contribute to the stem cell niche in the mammary gland. The recent discovery of markers that allow prospective isolation of mammary stem cells in mice should facilitate studies on the role of macrophages in regulating stem cell behaviour.[18–20]

SIMILARITIES BETWEEN BREAST DEVELOPMENT AND TUMORIGENESIS

Key features of malignant cells are their ability to invade surrounding tissues, resist apoptosis and induce new vessel formation. Breast development and maturation during pregnancy involves all of these processes. During puberty, epithelial cells invade the surrounding stroma to form a mature breast. During pregnancy and lactation, the increase in cell number and metabolic activity is a strong stimulus for angiogenesis.[21]

Vascular endothelial growth factor-A (VEGF-A) is a key regulator of angiogenesis. Hypoxia is an important stimulus for VEGF-A release from both normal mammary fibroblasts and tumour fibroblasts.[22] Steroid hormones may also play a role in VEGF-A release with significantly higher expression of this growth factor in breast tissue in premenopausal than postmenopausal women.[23] Multiple cancer cell lines also overexpress VEGF-A and there is growing evidence of the efficacy of anti-VEGF therapy (such as the anti-VEGF monoclonal antibody, bevacizumab) in the treatment of metastatic breast cancer.[24,25]

TUMOUR-ASSOCIATED MACROPHAGES IN BREAST CANCER

In 1863, Virchow was the first to notice the presence of inflammatory cells within a malignancy. He thought that this reflected the origin of malignancy within chronically inflamed tissues.[26] Subsequent evidence has linked tumour growth to myelopoiesis and the accumulation of circulating immature myeloid cells (also known as myeloid suppressor cells).[27] These cells are thought to play an immunosuppressive role by overwhelming the adaptive immune system with improper antigen-presenting cells. In mouse models these immature myeloid cells have been shown to differentiate into tumour-associated macrophages (TAM).[28]

Macrophages are recruited to tumours by various signals. One such signal is monocyte chemotactic protein-1 (MCP-1) expressed by tumour cells as well as endothelial cells and fibroblasts. Increased MCP-1 expression has been correlated to TAM accumulation in breast cancer.[29] MCP-1 expression also correlates to early relapse as well as with VEGF expression, suggesting that it may have a pro-angiogenic role.

Angiogenesis is an essential step for a malignant deposit to survive in a distant site and develop into a clinically significant metastasis. In primary tumours, microvessel density has been related to both disease-free and overall survivals.[30] A key factor in stimulating angiogenesis is the presence of macrophages within the tumour. Circulating monocytes are recruited to the tumour by chemotactic factors, including CSF-1, IL-4, IL-6, IL-10 monocyte growth factor, TGF-β and prostaglandin-E2. These factors allow the tumour to 'immunoedit'[31] the maturing monocyte to become a tumour-associated macrophage (Fig. 2). TAM have suppressed antigen presenting ability,[32] secrete EGF,[33] TNF-α,[34] VEGF[35] and bFGF[36] in response to tumour hypoxia and other factors, and thereby promote neoplasia and angiogenesis. The release of IL-10 by both the tumour and TAM blunts the antitumour effects of cytotoxic T cells.[37]

Tumour-associated macrophages in breast cancer have been associated with poor prognosis. A relationship has been shown between tumour macrophage number and both relapse-free and overall survivals.[36,38] Plasma levels of CSF-1 have also been correlated to poor prognosis where patients with locally advanced or metastatic breast cancer had higher mean levels of CSF-1 than patients with *in situ* carcinoma.[39] The association between TAM and reduced survival has also been shown in renal cell carcinoma,[40] bladder cancer,[41] prostate cancer[42] and lymphoma.[43] The significance of peritumoral macrophages in colon cancer is less clear; however, a recent study correlated serum M-CSF levels to poor prognosis.[44]

Further evidence for the role of macrophages in tumorigenesis exists in mouse models. Transgenic mice expressing the potent Polyoma virus middle T oncoprotein in the mammary gland develop mammary carcinoma. Lin *et al.* showed a significantly slower progression of premalignant lesions to malignancy as well as reduced formation of lung metastases when these mice were depleted of macrophages.[45] Real-time live animal imaging using multiphoton microscopy showed tumour cells invading the vasculature in tandem with perivascular macrophages, stressing a role for these cells in the intravasation of tumour cells and subsequent metastasis.[46]

Further evidence of the role of macrophages in tumorigenesis is seen in mouse tumours, which overexpress macrophage-stimulating protein (MSP). MSP was originally identified as a serum protein that elicited macrophage chemotaxis and activation. Overexpression of MSP has been associated with increased frequency of metastasis (particularly to bone) and reduced the time to metastasis.[47] Coordinate overexpression of MSP together with membrane-type serine protease 1 and macrophage-stimulating 1 receptor was also found to be an independent prognostic factor for overall survival.

THERAPEUTIC IMPLICATIONS

Tumour-associated macrophages present both a prognostic and therapeutic target in breast cancer and other malignancies. The growing evidence of the role that TAM play in promoting angiogenesis and metastasis as well as their immunosuppressive activities provides good reason for targeting these cells in anticancer therapy. A recently published strategy has used a DNA vaccine against legumain (a protein overexpressed on TAM) to induce a $CD8^+$ T-cell response against TAM suppressing angiogenesis and tumour growth and metastasis in a mouse model.[48] An alternative strategy might be to exploit the tumours' chemotactic stimuli to macrophages. *In vivo* experiments have shown that human macrophages, transduced with an adenoviral vector, are able to infiltrate tumour deposits and result in tumour cell death.[49]

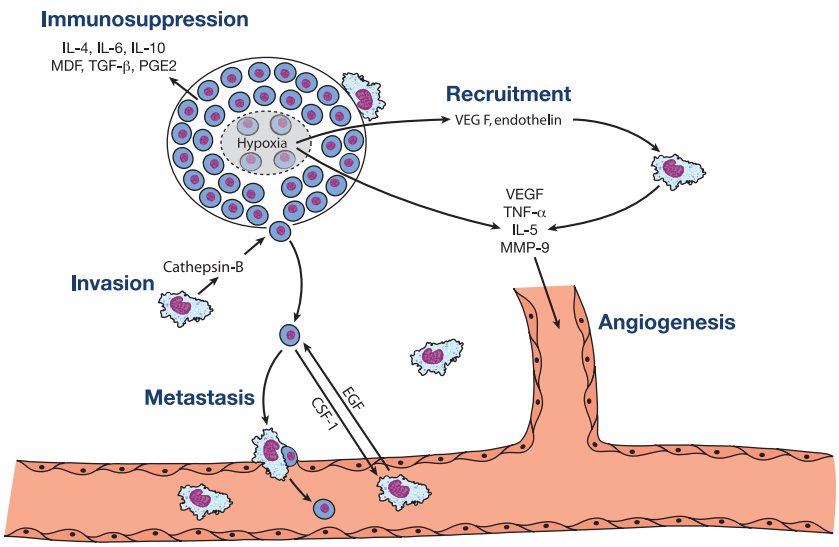

Fig. 2. Macrophages have pleiotropic roles in tumour growth and metastasis: *Recruitment* – vascular endothelial growth factor and endothelin released from hypoxic areas of tumours recruit circulating macrophages; *Angiogenesis* – factors released by the hypoxic milieu and the recruited macrophages promote neoangiogenesis; *Invasion* – macrophages play a central role in aiding the tumour cells to break through the basement membrane; *Metastasis* – cellular cooperation allows tumour cells to pass into the circulation in direct apposition to macrophages; *Immunosuppression* – numerous factors released by tumours 'immunoedit' the recruited macrophages to restrict their innate immune functions. Adapted from Lewis and Pollard with permission.[30]

CONCLUSION

The precise role of macrophages in breast development and in the pathogenesis of breast cancer is not yet clear, but there is abundant evidence to implicate some of their pleiotropic functions in tumour progression. Further research is required to identify their exact role and interactions and gain insights into breast tumorigenesis that could lead to novel therapeutic strategies to target breast cancer.

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