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Inflammation, proteases and cancer

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ABSTRACT

Tumours are complex tissues composed of ever-evolving neoplastic cells, matrix proteins that provide structural support and sequester biologically active molecules, and a cellular stromal component. Reciprocal interactions between neoplastic cells, activated host cells and the dynamic micro-environment in which they live enables tumour growth and dissemination. It has become evident that early and persistent inflammatory responses observed in or around developing neoplasms regulates many aspects of tumour development (matrix remodelling, angiogenesis, malignant potential) by providing diverse mediators implicated in maintaining tissue homeostasis, e.g., soluble growth and survival factors, matrix remodelling enzymes, reactive oxygen species and other bioactive molecules. This review highlights recent insights into the role of chronic inflammation associated with cancer development and examines proteolytic pathways activated by infiltrating leukocytes during neoplastic programming of tissues.

1. Introduction

The current predominating view of cancer is as a disease involving irreversible genomic change: changes encompassing single mutations in specific genes or alteration, amplification or loss of vast regions of the genome.[1] A unifying concept of this property of cancer is that dominant gain-of-function and recessive loss-of-function alterations in critical gate-keeper genes, e.g., oncogenes and tumour suppressor genes, have been identified in virtually every form of human cancer and are believed to be at the root of initiating neoplastic programs of growth within tissues. The sheer number of genes identified in the past 25 years harbouring such genomic alterations might suggest that random genetic mutations underlie cancer development. However, upon closer inspection, it is clear that while a diversity of mutated genes exist in cancer cells,[1,2] it is likely that a much smaller number of critical physiological pathways, when either chronically enabled or disabled, effects overall neoplastic risk.[3,4] Thus, genomic alterations affecting *intrinsic* cellular programs, e.g., cell cycle check-point control, programmed cell death, differentiation, metabolism and cell adhesion, in combination with epigenetic alterations affecting *extrinsic* programs, such as immune response, matrix metabolism, tissue oxygenation and vascular status, underlie human cancer development.[5,6]

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2. Immune response and cancer

The frequent presence of inflammatory cell infiltrates in tumours has been recognised for over a century,[7] although mechanistically understanding of their precise role in tumour development has been elusive until recently. Inflammatory cell infiltrates in pre-malignant tissues and tumours can be differentially composed of diverse leukocytic populations, adaptive and/or innate, depending upon the stage of tumour development and the organ micro-environment.

Innate immune cells, e.g., granulocytes (neutrophils, basophils and eosinophils), dendritic cells (DCs), macrophages, natural killer cells (NK cells) and mast cells, are prominent components of pre-malignant and malignant tissues (Fig. 1)[5,8] and functionally contribute to cancer development largely due to their release of potent soluble mediators that regulate cell survival and proliferation, angiogenesis, tissue remodelling, metabolism and genomic integrity. In a homeostatic context, when tissues are wounded or exposed to a chemical irritant, inflammatory cells are involved in removing damaged cells by induction of cell death and phagocytic pathways, as well as in enhancing matrix metabolism and cell proliferation to facilitate tissue regeneration or wound healing. Proliferation and inflammation subside after the assaulting agent is removed or the repair completed. In contrast, sustained proliferation of 'initiated/mutant' cells in environments rich in inflammatory cells, growth/survival factors, activated stroma, and DNA damage promoting agents, potentiates and/or 'promotes' neoplastic risk.[5,9–11] Individuals suffering from chronic inflammatory disorders harbour a greatly increased risk of cancer development,[5,6] largely due to the pro-growth environment generated by activated inflammatory cells. In addition, many clinical studies have reported the abundance of innate immune cells, in particular mast cells and macrophages, in human tumour samples and correlated their presence with either angiogenesis or clinical outcome.[6]

Macrophages are differentiated monocytes that originate from bone marrow and differentiate upon extravasation from the haematogenous vasculature.[12] They are recruited to sites of tissue injury, inflammation or proliferation by specific chemokines, e.g., monocyte chemotactic protein (MCP)-1 as well as various polypeptide growth factors.[13] Peri-tumoural accumulation of macrophages correlates with blood vessel density in a wide variety of tumours,[12] believed to be due largely to the myriad of proteases, growth factors and angiogenic factors produced by macrophages and then utilised by neoplastic cells to enhance their eventual development into cancers. In elegant work using mice lacking colony-stimulating factor (CSF-1), Pollard and colleagues demonstrated how macrophages contribute to later stages of malignant progression in the murine polyoma virus middle T oncogene model (PyMT) of mammary tumour development.[14] In more recent studies, Pollard and colleagues have described the existence of a reciprocal relationship between macrophages expressing CSF-1 receptor and epidermal growth factor (EGF) with tumour cells expressing EGF receptor and CSF-1 that together promotes metastasis of malignant tumour cells.[15] Tumour hypoxia may play a role in this relationship given the association between tumour-associated macrophages (TAMs) and hypoxic areas of tumours.[16] A number of recent studies have shown that macrophages respond to the levels of hypoxia found in tumours by up-regulating transcription factors such as hypoxia-inducible factors 1 and 2, which in turn activate a broad array of mitogenic, pro-invasive, pro-angiogenic, and pro-metastatic genes[17] and thus may explain why high numbers of TAMs correlate with poor prognosis in various forms of cancer.[12]

Macrophages, however, are not the only innate immune cells exploited to a tumour's advantage – mast cells also play an important role. In a mouse model of squamous epithelial carcinogenesis, e.g., K14-HPV16 transgenic mice, where human papillomavirus type 16 early region genes are expressed in basal keratinocytes under the control of the keratin 14 promoter/enhancer,[18] genetic depletion of mast cells ($KIT^{W/}KIT^{WV}$) diminishes pre-malignant angiogenesis and reduces proliferation of keratinocytes and stromal fibroblasts, resulting in

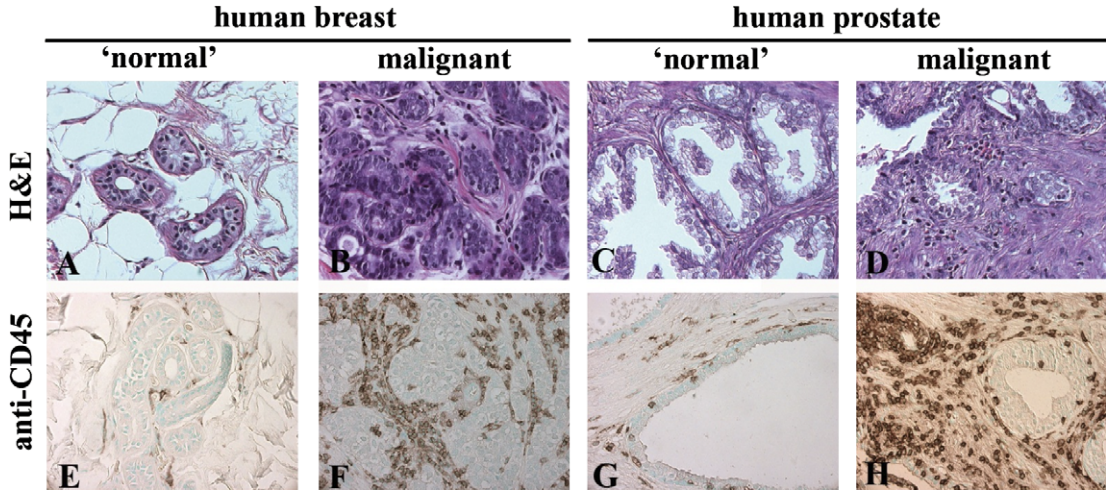

Fig. 1 – Human breast and prostate carcinoma development is accompanied by CD45$^+$ leukocyte infiltration. In haematoxylin and eosin (H& E) stained tissue sections of (A, C) 'normal' or (B, D) malignant human breast and prostate tissues, it is difficult to discern the presence of infiltrating leukocytes. By contrast, immunodetection of CD45 (leukocyte common antigen) reveals limited presence of leukocytes in (E, G) 'normal' tissue as opposed to (F, H) malignant counterparts.

attenuated malignant progression.[19] Moreover, in K14-HPV16 mice genetic elimination of mature T and B lymphocytes limits neoplastic progression to development of benign hyperplasias that fail to recruit innate immune cells, including mast cells, granulocytes and a majority of other $CD45^+$ leukocytes, into pre-malignant tissue, and as a result reduces carcinoma incidence from 47% to 6%.[20] Adoptive transfer of $CD19^+B220^+$ B cells or serum from K14-HPV16 mice into T and B cell-deficient/HPV16 mice is sufficient to restore innate immune cell infiltration into pre-malignant tissue and to reinstate necessary parameters for full malignancy, e.g., chronic inflammation, angiogenic vasculature and hyperproliferative epidermis.[20] These findings support a model in which activation of peripheral adaptive immune responses, specifically B lymphocytes, and soluble molecules in serum are required for establishing chronic inflammatory states, composed of innate immune cells, that promote de novo epithelial carcinogenesis, and support the concept that oncogene expression in 'initiated' cells alone is not sufficient for full malignant progression. Instead, additional signals provided by adaptive and innate immune cells are required for elaboration of the malignant state, and this suggests that pharmacological interventions targeting B lymphocytes and/or recruitment of innate immune cells towards pre-malignant tissue represents a viable cancer chemopreventative strategy.

Tumour-associated neutrophils have also been identified as innate immune cells that enhance the in vitro invasive and in vivo metastatic potential of syngeneic tumour cells by facilitating invasion across basement membranes.[21] Neutrophils are also implicated in carcinogenesis through their generation of reactive oxygen and nitrogen species,[22] a potential mechanism for the significant association between chronic inflammatory diseases and increased cancer risk.

Another intriguing role for host inflammatory involvement in tumourigenesis was proposed recently.[23] During chronic gastric inflammation induced in mice, inflammatory infiltrates harbour not only mature differentiated cells typically associated with an immune response, but also bone marrow stem cells that become incorporated into the glandular epithelium of the stomach at sites of eventual tumour formation. Furthermore, a correlation between release of the E2F1 transcription factor from the retinoblastoma protein in colonic epithelial cells was recently appreciated in a murine model of ulcerative colitis and in human tissue with similar pathology.[24] Subsequent transcription of E2F1 target genes (e.g., proliferating cell nuclear antigen and cyclinD1) and promotion of cell cycle progression and proliferation provides another likely link between chronic inflammation and cancer. Whether these processes occur at other sites of chronic inflammation and, more importantly in human disease, remains to be determined.

Since immune cells exist as part of an organism's defence mechanism against foreign bodies, it may be expected that the true goal of inflammatory cell recruitment to a tumour is to eradicate the aberrant mass. It therefore seems likely that many immune cells will have anti-tumourigenic functions, but these must be balanced against the pro-tumourigenic properties discussed above. Tissue specificity must also be considered when determining whether inflammatory cells are ultimately pro- or anti-tumourigenic. This is evident when comparing the association between prognosis and macrophage infiltration in breast and colon tumours. In breast cancer, significant infiltration is associated with a poor prognosis, whereas in colon cancer, the few studies performed to date suggest the opposite, i.e., that macrophage infiltration is a good sign.[12] The ability of tumours to suborn a defence mechanism to further its own development is a sign of the rapid evolution process that occurs within tumours and also one of the many reasons why cancer is difficult to treat effectively.

2.1. *Inflammatory cell-derived mediators: proteases*

Tumour infiltrating leukocytes also indirectly contribute to tumour development by production of extracellular proteases.[25–30] Numerous studies have documented increased expression of matrix metalloproteinases (MMPs) in human malignant tissue, often correlating with poor prognosis.[31] MMPs regulate tissue homeostasis and disease pathogenesis via pleiotropic biological effects, including remodelling of soluble and insoluble extracellular matrix (ECM) components and cell–cell and cell–matrix adhesion molecules.[31] In both human and mouse models of cancer development, whereas some MMPs are produced by epithelial cells, activated stromal cells, e.g., fibroblasts, vascular cells, and in particular innate immune cells, are the major sources of MMPs.[31]

Several mechanistic studies have reported that leukocyte-derived MMPs functionally contribute to neoplastic progression.[25,26,28,32] For example, we have previously reported that tumour incidence and growth in the K14-HPV16 mouse model of de novo epithelial carcinogenesis is reduced in the absence of MMP-9.[25,33] Characteristics of neoplastic development were restored by reconstitution of MMP-9-deficient/K14-HPV16 mice by adoptive transfer of wild-type bone marrow-derived cells, indicating that inflammatory cells functionally contribute to de novo carcinogenesis at least in part by their deposition of MMP-9 into the neoplastic micro-environment.[25,34] While genetic elimination of MMP-9 or amino-bisphosphonate-mediated blockade of MMP-9 production by macrophages significantly reduces cancer development in HPV16 mice, infiltration of neoplastic tissue by immune cells is unperturbed by MMP-9 absence,[33,34] indicating that one mechanism whereby inflammation potentiates cancer risk is by local delivery of MMP-9.

Other experimental mouse models of cancer development have similarly identified MMP-9 as a key inflammatory cell-derived mediator of tumour-associated angiogenesis.[27,28,35] During pancreatic Islet carcinogenesis for example, Bergers and colleagues determined that MMP-9, produced predominantly by macrophages, regulates angiogenesis by mobilising ECM-sequestered vascular endothelial growth factor (VEGF) and stimulating vascular endothelial cell proliferation and subsequent angiogenesis.[27] Processing of pro-growth factors is not a unique property of MMP-9, in fact several MMP family members are known to possess this property, some of which also regulate acute inflammation via their ability to process chemokines.[36] MMP-7 produced by osteoclasts has emerged as a significant regulator of prostate cancer bone metastases by virtue of its ability to process receptor activator of NF kappa B ligand (RANKL) and induce osteolysis.[37]

Since osteoclasts and macrophages are similarly derived from monocyte precursors, it will be interesting to determine if bisphosphonate therapy attenuates MMP-7 production by osteoclasts similarly to its inhibition of macrophage MMP-9 during cervical carcinogenesis.[33] Bisphosphonates are known significantly to reduce the incidence of skeletal-related events during breast cancer metastases to bone;[38] thus, perhaps the mechanisms by which this is achieved is by monocyte blockade of MMP production and subsequent inhibition of skeletal complications resultant from bone metastases.

MMPs are also thought to promote tumour cell survival by conferring protection against apoptotic cell death. For example, MMP-7 sheds membrane-bound Fas ligand (FasL), resulting in production of soluble FasL that significantly lowers the ability to trigger apoptosis via the Fas receptor pathway.[39] MMP-7 also confers protection from apoptosis by cleaving the heparin-binding EGF precursor (HB-EGF) from the cell surface resulting in generation of signals conferring protection from apoptosis by binding of mature active form of HB-EGF to both the ErbB1 and ErbB2 receptors.[40]

MMPs, besides promoting tumour progression via these diverse mechanisms, also exhibit anti-tumour functions. For example, male mice deficient for MMP-8 (collagenase-2) exhibit a significant increase in skin tumour incidence upon chemically induced carcinogenesis.[41] Tumour susceptibility is sex hormone dependent since ovarectomised MMP-8-deficient female mice also demonstrate a similar enhanced susceptibility to chemically induced skin carcinogenesis.[41] Moreover, treatment of MMP-8-deficient mice with tamoxifen, an oestrogen receptor antagonist, also resulted in increased skin carcinogenesis in females,[41] suggesting that loss of MMP-8 function, by either homozygous loss or MMP inhibition (natural or synthetic), enhances rather than reduces tumour susceptibility. Taken together, it is clear that MMP function extends well beyond ECM remodelling and, as a consequence of their diverse activities toward substrates, MMPs participate in many biological (e.g., embryogenesis, angiogenesis, endometrial cycling and wound healing) and pathological (e.g., cancer, arthritis and cardiovascular disease) processes by both positive and negative mechanisms.

In addition to proteolytic enzymes from the MMP family, other classes of intracellular and extracellular enzymes released by activated leukocytes have been identified as important mediators of neoplastic progression. Mast cell activation results in degranulation and release of (amongst many bioactive mediators) collagenase (MMP-1)[42] and two neutral serine proteases, chymase and tryptase.[43] Tryptase possesses mitogenic activities towards keratinocytes and fibroblasts via its ability to activate protease activated receptor (PAR)-2,[44,45] induces type I collagen expression by fibroblasts[44] as well as induces expression of the chemokine MCP-1 by monocytes and endothelial cells and subsequently stimulating monocyte/macrophage and granulocyte recruitment.[46–48] Chymase, on the other hand, does not possess direct mitogenic activities, but instead activates several pericellular enzymes, including proMMP-1, −2 and −9.[42,49–52] By evaluating skin, heart and lung tissues in mast cell protease-4 (chymase) homozygous null mice, a major role for chymase in regulating thrombin and fibronectin turnover was revealed, as was the necessity of chymase for activation of proMMP-9 to its zymogen form, and thus indicating that despite the fact that many enzymes can activate proMMP-9 in vitro, in vivo, chymase appears to be critical.[53,54] Moreover, chymase regulates the rennin-angiotensin pathway by generating angiotensin II from angiotensin I, which it can also liberate via cleavage of angiotensinogen.[55,56] Angiotensins are significant chymase substrates, since angiotensin I is a potent fibroblast mitogen and induces expression of diverse collagens, laminins and fibronectin via activation of the angiotensin 2 receptor.[56] Chymase also possesses indirect pro-angiogenic activities in addition to modulation of angiotensins via regulating release of sequestered VEGF from the matrix following cleavage of an as yet unidentified substrate.[57]

A major role for cysteine cathepsin proteases, produced by leukocytes and epithelial cells, as important mediators of cancer development, has also been recently appreciated.[58] While many cysteine cathepsins are lysosomal proteases, they are known to be involved in remodelling of ECM, to regulate cellular proliferation and death, to activate tumour angiogenesis, to promote invasion and metastasis of tumour cells, and to regulate inflammatory and immune responses in tissues.[59] Joyce and colleagues recently demonstrated the association of increased cathepsin activity with angiogenic vasculature and invasive fronts of carcinomas during tumourigenesis in transgenic mouse models of Islet cell and cervical carcinogenesis using activity-based chemical probes and in vivo imaging,[60] thus indicating that broad-spectrum cysteine cathepsin inhibitors may effectively block multiple biological aspects of tumour development, offering new therapeutic opportunities in anti-cancer therapy.

3. Paracrine signalling networks between leukocytes and neoplastic cells

On a molecular level, several studies have provided insight into which intracellular signalling pathways are co-opted in initiated neoplastic cells at-risk for cancer development. The pro-inflammatory transcription factor nuclear factor κB (NF-κB), a mediator of cell survival, proliferation and growth arrest, has been identified as an important molecule linking chronic inflammation and cancer.[61–63] Specific deletion of IKKκ – a key intermediary of NF-κB – in myeloid cells decreased carcinoma growth in a mouse model of colitis-associated cancer through reduced production of tumour-promoting paracrine factors.[62] In addition, examination of a mouse model of inflammation-associated hepatocellular carcinogenesis, similarly implicated activation of hepatocyte NF-κB via production of inflammatory cell-derived TNF-α.[61] These two mouse models reveal that the NF-κB pathway has dual actions in tumour promotion: first by preventing death of cells with malignant potential, and secondly by stimulating production of pro-inflammatory cytokines in cells of myeloid and lymphoid origin in tumour masses. Pro-inflammatory cytokines then signal to initiated and/or otherwise 'damaged' epithelial cells and promote neoplastic cell proliferation and enhance cell survival; thus, inflammatory cells in these contexts modulate gene expression extrinsically within neoplastic cells and favour proliferation and survival by paracrine regulation of NF-κB.

How then is bioavailability of important molecules such as TNF-α regulated? TNF-α is mainly synthesised by cells of the monocyte-macrophage lineage, including mast cells, macrophages, T-cells, natural killer cells, and neutrophils; however, epithelial cells are also known to upregulate TNF-α expression during malignancy.[64] TNF-α is expressed as a membrane-bound homotrimer that is proteolytically released (shed) by the metalloproteinase tumour necrosis factor α-converting enzyme (TACE/ADAM17) resulting in the release of the active C-terminal portion from the cell surface.[65] In homeostatic tissues, shedding of TNF-α facilitates rapid responses to tissue damage by activating both cell proliferation and cell death programs in 'damaged' tissues.[64] However, in neoplastic tissues, chronic bioavailability of TNF-α has been associated with enhanced invasive activities and survival of neoplastic cells.[64] Shedding of biologically active TNF-α is regulated by the endogenous metalloproteinase inhibitor TIMP-3.[66] TIMP-3 is sequestered at the cell surface by association with glycosaminoglycan chains of proteoglycans, especially heparan sulphate, and inhibits the shedding and bioavailability TNF-α by TACE,[67,68] and thus represents a rate-limiting step for both acute and chronic inflammation. During cancer development, cell-type specific expression of TIMP-3 appears to be critical as loss of TIMP-3 expression in activated stromal cells exacerbates inflammation, enhances angiogenesis and elicits rapid tumour development, whereas absence of TIMP-3 in neoplastic epithelial cells does not alter tumour latency, burden or potential.[69] The degree to which cell-type specific expression of TIMP-3 regulates NF-κB signalling pathways via TNF-α and TACE remains to be determined.

4. Conclusion

A vast body of evidence indicates that inflammatory leukocytes contribute to cancer development either directly via the release of vesicle-stored growth and survival factors and diverse proteolytic enzymes, or indirectly via the activation of cell signalling cascades as a result of altered pericellular matrix remodelling activity. Thus, chronic engagement of pro-inflammatory programs in pre-malignant tissues favours generation of a pro-growth environment that fosters cancer development. While undoubtedly complex, identifying the major mediators and pathways responsible for triggering inflammatory cell infiltration into 'damaged' tissue or their accumulation in (pre-)malignant tissues may provide therapeutic opportunities for prevention and treatment of cancer. The incredible efficacy of anti-inflammatory therapies[10,70] in chemoprevention argues for anti-inflammatory therapy at the earliest stages of neoplastic progression. Alternatively, should future anti-cancer strategies focus on regulating NF-κB activation, TNF-α bioavailability or metalloproteinase activity? In answering this question, it is important to point out that all organs are endowed with unique cell death and damage-response pathways that naturally invoke acute activation of innate immune cells. In skin, for example, keratinocyte cell death is by terminal differentiation.[71] Inhibiting NF-κB in keratinocytes promotes squamous cell carcinogenesis by reducing growth arrest and terminal differentiation of initiated keratinocytes[72] that proliferate in micro-environments where growth factors, matrix remodelling enzymes and reactive oxygen species produced by infiltrating inflammatory cells contribute to angiogenesis and keratinocyte DNA damage.[5] Similarly, hepatocytes exposed to carcinogens have a differential propensity towards hepatocellular carcinoma dependent upon the status of NF-κB in myeloid cells responding to hepatocyte damage.[73] On the other hand, blockade of TNF-α attenuates skin tumour formation.[74] Therapeutically regulating TNF-α however, must also be considered with care, as it too possesses opposing activities that are cell-type and environment-dependent.[75] Phase I clinical trials of TNF-α antagonists are currently underway in patients with advanced cancer – these may help us understand the complexities of these responses.[74,75] Similarly, expression and activity of MMPs varies by organ and in response to damage,[31] and while elimination or attenuation of MMP activity clearly evokes a survival advantage in immune competent mouse models of cancer development, efficacy of metalloprotease inhibitors in human clinical trials was disappointing at best.[76] So, what are the lessons we can learn from these failures? It is clear that special consideration must be given to understanding the stage of tumour progression where cytostatic agents targeting inflammatory mediators are likely to work alone, and where, when combined with standard debulking or cytotoxic therapies, an experimentally assessable advantage is provided. Mouse models that more closely mimic human cancers are rapidly becoming available and must be applied in a way that also recapitulates the presentation and current therapeutic approach to the corresponding human disease.

Conflict of interest statement

None declared.

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