The two faces of the inflammasome adaptor ASC in epithelial skin carcinogenesis

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ABSTRACT

The development of tumours is a multistep process during which cells acquire the capability to sustain proliferation, evade growth suppressors and/or resist cell death. One factor, which is increasingly recognised to influence tumour progression, is the inflammatory environment of the tumour. The responsible molecular mechanisms and signalling pathways are only beginning to emerge. One major pathway able to induce potent inflammation is the activation of the inflammasome and the subsequent secretion of the pro-inflammatory cytokines IL-1β and IL-18. Both these cytokines have been implicated in tumour-genesis/progression. However, evidence for the role of inflammasomes in this process is still scarce and mainly derived from murine colitis associated tumour models. In this short review we discuss current knowledge on the role of inflammasomes in epithelial cancer of the gut and skin with a special focus on the complex role of the inflammasome adaptor ASC in epithelial skin carcinogenesis.

Inflammasome activation in carcinogenesis

NLRs (nucleotide oligomerisation domain leucine rich repeat containing receptors) belong to the still increasing number of pattern recognition receptors (PRRs), with which our organism is able to sense and react to danger. Some members of the NLR family are able to form large protein complexes that lead to the activation of the cystein protease caspase-1 and the subsequent release of active IL-1β and IL-18 from the cell (1, 2). These complexes are now commonly referred to as inflammasomes. To date five distinct inflammasomes have been extensively described either consisting of NLRP1, NLRP3, NLRP6, NLRC4 or the HIN-family member AIM2. While their role in numerous inflammatory pathologies, including gout and periodic fever syndromes is well established (3-5), data on inflammasome involvement in tumour development/progression is so far mainly restricted to colitis associated cancer.

In general, chronic inflammatory processes seem to be a driving force in the etiology of numerous tumours (6). These include gastric neoplasms (*Helicobacter pylori* infection), hepatocellular carcinoma (Hepatitis B or C, alcoholism and obesity), colorectal cancer (inflammatory bowel disease) and squamous cell carcinoma (UV-radiation or papilloma virus infection). The two inflammasome-dependent cytokines IL-1β and IL-18, in particular, have both been implicated in inflammation induced gastric tumourigenesis (7), FGFR1 driven breast cancer (8) and 3-methylcholanthrene-induced subcutaneous fibrosarcoma (9). The capability of IL-1β to drive tumour formation in those models is mainly associated to the recruitment of myeloid cells and the subsequent activation of NF-κB downstream of the IL-1R1 (10). Extensive evidence for a role of IL-18 in tumour development/progression, so far, only exists from colitis-associated cancer models (11, 12). Unlike IL-1β, however, it seems to have a protective function in those settings by regulating the integrity of the gut epithelium. The invasion of commensal bacteria as a result of epithelium disintegrity in IL-18-deficient mice leads to chronic inflammation and tumour development. A similar phenotype exists in caspase-$1^{-/-}$ mice where the administration of recombinant IL-18 reduces tumour formation (13, 14). The regulation of tissue homeostasis by IL-18 is dependent on the NLRP3 and NLRP6 inflammasomes. The activation of this signalling cascade results in the down-regulation of IL-22 binding protein

(IL-22BP) thereby increasing IL-22 activity and as a result epithelial barrier repair (11). This data from animal models is supported by clinical observations in Crohn's patients where IL-18 expression is increased in colonocytes (15).

While the tumour-protective effect of caspase-1 and IL-18 in the gut is very well established, the inflammasome responsible for these phenotypes is still under debate. Several studies have attempted to address this question. What complicates this matter is that not only the myeloid cells present in the gut mucosa but also the epithelium cells themselves are capable of inflammasome activation. Furthermore, it seems that inflammasome activation can have different effects depending on the cell type and the specific inflammasome that is activated. For instance, NLRP3 activation in myeloid cells has been shown to support gut barrier integrity (14, 16), while NLRC4 expressed in epithelial cells can influence epithelial cell proliferation and hence, tumour formation (17). Moreover, the epithelial cell-specific NLRP6 inflammasome is able to regulate the microbial flora of the gut and reduce the risk of colitis (18). These studies indicate how complex a system the gut is when it comes to dissecting the role of inflammation and tissue homeostasis/tumour development. Nonetheless, what has become clear in recent years is that inflammasomes do play a major role in maintaining epithelial integrity. Numerous studies point to a role of inflammasomes in this process thereby protecting against chronic inflammation and the development of tumours.

The multiple roles of the inflammasome adaptor ASC in epithelial skin carcinogenesis

Similar to the gut epithelium, the skin represents another organ, which is constantly exposed to the external environment. The epidermis forms a continuous barrier of keratinocytes in order to protect the host from exposure to microbial products and danger signals and the subsequent induction of inflammation. Unlike in the gut, however, IL-1β signalling, rather than IL-18, seems to play a major role in the skin. It has been shown to be involved in numerous skin pathologies, including contact dermatitis, acne and psoriasis (19). In the context of tumour development it is important to note that the major risk factor for the development of squamous skin carcinoma, UV-radiation, is a powerful inducer of IL-1 (20). And once more reflecting the situation in the gut, not only the myeloid cells present in the skin are inflammasome competent but also the keratinocytes themselves are a source of inflammasome dependent cytokines (20). Recent data directly implicates NLRP3 in promoting inflammation-induced skin cancer (21). Since NLRP3 is not expressed in murine keratinocytes (22), this study might suggest that the major source of IL-1 driving skin carcinogenesis are the myeloid cells infiltrating the skin rather than keratinocytes. Using the same mouse model, a recent study does confirm this observation. Mice lacking the common inflammasome adaptor ASC specifically in the macrophage/neutrophil compartment show a similar phenotype as NLRP3 and caspase-1 deficient mice, as they are partially protected from tumour development (23). On the other hand, animals lacking ASC expression specifically in keratinocytes do develop more tumours than their wild-type controls (23). This result would implicate ASC as a tumour suppressor. Indeed the same study provided evidence for an inflammasome independent role of ASC in regulating cell-cycle progression in keratinocytes (23). The tumour suppressive role of ASC has been hypothesised since the original description of the ASC gene (24). The ASC gene is down-regulated in tumour tissues due to the methylation of its promoter (25). This has been first disrobed in breast cancer and has since been repeated in numerous other tumour tissues (26-32). Our results do add to this list, as we could show the down-regulation of ASC in squamous skin carcinoma (23). In this system the reduction of ASC expression is not a side-effect of keratinocyte proliferation as ASC expression remains intact in psoriatic lesions (23). Therefore, the lack of ASC expression occurs specifically in the context of tumour development and strengthens its implication as a tumour-suppressor. Similar results were obtained in melanoma. Human melanoma cells contain a constitutively active inflammasome, thereby mediating autoinflammation associated with tumourigenesis (33). Moreover, Fujita et al. also observed that down-regulation of ASC expression in primary melanoma tumour tissue reduced cell death, increased cell viability, and enhanced tumourigenesis (34).

In analogy to colitis-associated cancer, the function of inflammasomes and/or inflammasome components in cutaneous tumourigenesis is complex and involves tissue specific functions, which in some cases exhibit diametrically opposing effects.

Conclusions

The multiple and sometimes opposing roles of inflammasomes in tumourgenesis make it impossible, at this stage, to form an overall conclusive model of inflammasome function in cancerogenesis. This complexity seems to be a common feature in the relationship between inflammatory mediators and tumour formation/progression, *e.g.* TNF dependent squamous cell carcinoma *versus* TNF induced senescence (35, 36). In case of the inflammasome, it has been convincingly shown that inflammasome activation increases chemotherapeutic regimens; but also that it promotes tumour growth and impairs tumour vaccines (37-41).

Thus, with our current knowledge, blocking inflammasome activation might result in unwanted and unforeseen consequences regarding tumour progression. The outcome seems to be dependent on the type of tissue in which inflammasomes are activated and the stage of tumourigenesis.

Recent data has shown that ASC possesses inflammasome-independent roles in the regulation of tumour progression. Since its expression is down-regulated specifically in tumour tissues by promoter methylation, it might represent a saver therapeutic target for tumour treatment compared to inflammasome blockade (42). Indeed, the use of de-methylating drugs has already

shown efficacy in case of haematological and epithelial human cancer cells (43) and was shown to reverse drug resistance in methylation-silenced ASC in bladder carcinoma (44). Besides its potential as a therapeutic target, it would be intruiging to investigate the possible correlation of ASC down-regulation with different tumour stages as well as aggressivness. This could poteintially lead to the exploitation of ASC as a biomarker for tumours and drug effectiveness.

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