Neutrophils fan cancer's flames

Stefanie K Wculek & Ilaria Malanchi

A new study published in this issue of *The EMBO Journal* looks into the cross-talk between inflammation and cancer (Antonio *et al*, 2015). By using a zebrafish melanoma model, the authors reveal that neutrophils recruited at the wound site directly interact with cells undergoing oncogenic transformation and provide them with a paracrine proliferative support. Importantly, the authors demonstrate the clinical relevance of this association, showing that neutrophil infiltration has an independent prognostic value for human melanoma. This study reinforces the notion that inflammation flames carcinogenesis, which might have important implications for the improvement of antitumour therapies.

See also: N Antonio *et al* (September 2015)

Genetic mutations are the initial spark of tumour initiation and the driving force in tumour progression; however, dramatic changes in the micro-environment accompany and fuel cancer cell growth. Indeed, a variety of infiltrating cell types impact the whole tumour activity and contribute to generate cancer cell heterogeneity which is required for tumour progression (Kreso & Dick, 2014). Inflammatory cells are one of the main components within the tumour microenvironment having a recognized pivotal role in tumour promotion, progression and metastatic spreading (Grivennikov & Karin, 2010). Hence, cancer-associated inflammation represents an important target for the development of novel anticancer therapies.

Neutrophils are innate immune cells and essential during tissue damage and wound healing processes. They are quickly mobilized to the damaged tissue and are required to protect against invading pathogens, build and modulate immune responses. In recent

years, growing evidence shows neutrophil involvement in various physiologic processes and their functional relevance during tumorigenesis has become an active field of investigation. In cancer patients, high tumour-associated neutrophil density correlates with unfavourable outcome; therefore, mechanistic studies on the role of neutrophils in tumorigenesis are critical (Shen *et al*, 2014). Interestingly, neutrophils are reported to display both antitumour and pro-tumour responses according to different signals within the tumour microenvironment (Fridlender *et al*, 2009). Indeed, three phenotypically distinct sub-pools of neutrophils with conflicting functions have been identified in the circulation of tumour-bearing mice and cancer patients, indicating high neutrophil plasticity (Sagiv *et al*, 2015). The main antitumour activity of neutrophils is linked to their cytotoxicity, an example of which was recently reported in melanoma where a sub-pool of tumour-associated neutrophil promotes cancer cell killing via nitric oxide release (Finisguerra *et al*, 2015). Conversely, neutrophils have been extensively reported to promote tumour growth by influencing the tumour microenvironment using mechanism such as the promotion of angiogenesis (Nozawa *et al*, 2006; Shojaei *et al*, 2008) or the creation of a “safe” milieu where more immature neutrophils alongside with macrophages suppress antitumour immune responses (Katoh *et al*, 2013). However, evidence of neutrophil plasticity predicts that many novel neutrophil-mediated functions are yet to be discovered.

In this issue of *The EMBO Journal*, Antonio and colleagues made an important contribution to the debated role of neutrophils in tumorigenesis by unravelling their novel direct pro-tumorigenic activity during wound healing in a zebrafish model of melanoma (Antonio *et al*, 2015). Here, the expression of a Ras^{G12V} oncogene in skin

leads to the development of invasive melanoma. Using this model, which enables *in vivo* imaging to study the dynamics of cell growth, the laboratory has previously shown that myeloid cells can provide direct trophic support to neoplastic cells during tumour initiation (Feng *et al*, 2012). Antonio and colleagues now report that, in the context of wound healing, neutrophils, but not macrophages, boost pre-neoplastic cell's proliferative activity via a direct paracrine mechanism (Fig 1).

Post-surgery wound healing-associated inflammation might influence the tumorigenic ability of cancer cells remaining in the tissue; therefore, the investigation of tumour-promoting effects of wound healing is especially relevant in the context of tumour relapses after surgical tumour removal. By taking advantage of *in vivo* imaging of zebrafish larvae, Antonio *et al* (2015) mimicked the consequences of cancer surgery through generation of a wound and directly investigated the consequences of the recruitment of inflammatory cells on the neoplastic cells in the wound proximity. Results showed that hydrogen peroxide released by the damaged tissue attracted a great number of neutrophils, but, in contrast to the physiologic response where neutrophils remained in the proximity of the wound, in the presence of the near pre-neoplastic cells, neutrophils were diverted from the wound site and engaged in direct interaction with growing cancer cells. Importantly, results indicated that this interaction provides pre-neoplastic cells with a proliferative advantage leading to an increase in clones size. Moreover, the authors provide evidence that the direct neutrophil-mediated pro-tumorigenic activity described in zebrafish might be relevant for human melanoma. They report that neutrophils are predominantly infiltrating human melanomas with ulcerative lesions and their

Figure 1. Neutrophils attracted by an adjacent wound foster outgrowth of pre-neoplastic cell clones. In a zebrafish melanoma model, Antonio *et al* (2015) show that neutrophils, which are recruited to the site of injury through release of hydrogen peroxide (H₂O₂), are diverted into the proximity of pre-neoplastic cells. Via mechanisms involving release of prostaglandin E₂ (PGE₂), neutrophils drive proliferation of pre-neoplastic cells resulting in clone establishment.

presence correlates with increased proliferation. Significantly, Antonio *et al* (2015) show that neutrophil infiltration in melanoma is functionally important for disease progression since it refines the known prognostic value of ulceration and represents an independent prognostic indicator.

Collectively, the work by Antonio and colleagues corroborates the contribution of neutrophils to the tumorigenic process by defining a pro-tumorigenic role directly targeting cancer cells. The authors found immune-cell-derived PGE₂ to be part of the paracrine mediators of wound-induced pre-neoplastic proliferation in zebrafish (Fig 1), but results show that other signals are also involved. What would be fascinating to define are the precise mechanisms of cancer

cell–neutrophil interaction. On the ground of Antonio *et al*'s findings, future studies need to concentrate on the dissection of these neutrophil-mediated pro-tumour activities, which will provide novel targets to improve the efficacy of antitumour therapies.

References

- Antonio N, Bønnelykke-Behrndtz ML, Ward LC, Collin J, Christensen IJ, Steiniche T, Schmidt H, Feng Y, Martin P (2015) The wound inflammatory response exacerbates growth of pre-neoplastic cells and progression to cancer. *EMBO J* 34: 2219–2236
- Feng Y, Renshaw S, Martin P (2012) Live imaging of tumor initiation in zebrafish larvae reveals a trophic role for leukocyte-derived PGE₂. *Curr Biol* 22: 1253–1259
- Finisguerra V, Di Conza G, Di Matteo M, Serneels J, Costa S, Thompson AAR, Wauters E, Walmsley S, Prenen H, Granot Z, Casazza A, Mazzone M (2015) MET is required for the recruitment of anti-tumoural neutrophils. *Nature* 522: 349–353
- Fridlender ZG, Sun J, Kim S, Kapoor V, Cheng G, Ling L, Worthen GS, Albelda SM (2009) Polarization of tumor-associated neutrophil phenotype by TGF- β : “N1” versus “N2” TAN. *Cancer Cell* 16: 183–194
- Grivennikov SI, Karin M (2010) Inflammation and oncogenesis: a vicious connection. *Curr Opin Genet Dev* 20: 65–71
- Katoh H, Wang D, Daikoku T, Sun H, Dey SK, DuBois RN (2013) CXCR2-expressing myeloid-derived suppressor cells are essential to promote colitis-associated tumorigenesis. *Cancer Cell* 24: 631–644
- Kreso A, Dick JE (2014) Evolution of the cancer stem cell model. *Stem Cell* 14: 275–291
- Nozawa H, Chiu C, Hanahan D (2006) Infiltrating neutrophils mediate the initial angiogenic switch in a mouse model of multistage carcinogenesis. *Proc Natl Acad Sci USA* 103: 12493–12498
- Sagiv JY, Michaeli J, Assi S, Mishalian I, Kisos H, Levy L, Damti P, Lumbroso D, Polyansky L, Sionov RV, Ariel A, Hovav A-H, Henke E, Fridlender ZG, Granot Z (2015) Phenotypic diversity and plasticity in circulating neutrophil subpopulations in cancer. *Cell Rep* 10: 562–573
- Shen M, Hu P, Donskov F, Wang G, Liu Q, Du J (2014) Tumor-associated neutrophils as a new prognostic factor in cancer: a systematic review and meta-analysis. *PLoS ONE* 9: e98259
- Shojaei F, Singh M, Thompson JD, Ferrara N (2008) Role of Bv8 in neutrophil-dependent angiogenesis in a transgenic model of cancer progression. *Proc Natl Acad Sci USA* 105: 2640–2645