Morphed and moving:
TNFα-driven motility promotes cell dissemination through MAP4K4-induced cytoskeleton remodeling

Min Ma1,2 and Martin Baumgartner1,*

1 Neuro-Oncology Laboratory, Experimental Infectious Diseases and Cancer Research, University Children’s Hospital Zürich, 8008 Zürich, Switzerland.
2 Graduate School for Cellular and Biomedical Sciences, University of Bern, Switzerland.
* Corresponding Author: Martin Baumgartner, Neuro-Oncology Laboratory, Experimental Infectious Diseases and Cancer Research, August Forel Strasse 1; 8008 Zürich, Switzerland; Tel: +41 44 634 88 51; E-mail: martin.baumgartner@kispi.uzh.ch

Cell dissemination from an initial site of growth is a highly coordinated and controlled process that depends on cell motility. The mechanistic principles that orchestrate cell motility, namely cell shape control, traction and force generation, are highly conserved between cells of different origins. Correspondingly, the molecular mechanisms that regulate these critical aspects of migrating cells are likely functionally conserved too. Thus, cell motility deregulation of unrelated pathogenesis could be caused and maintained by similar mechanistic principles. One such motility deregulation disorder is the leukoproliferative cattle disease Tropical Theileriosis, which is caused by the intra-cellular, protozoan parasite Theileria annulata. T. annulata transforms its host cell and promotes the dissemination of parasite-infected cells throughout the body of the host. An analogous condition with a fundamentally different pathogenesis is metastatic cancer, where oncogenically transformed cells disseminate from the primary tumor to form distant metastases. Common to both diseases is the dissemination of motile cells from the original site. However, unlike metastatic cancer, host cell transformation by Theileria parasites can be reverted by drug treatment and cell signaling be analyzed under transformed and non-transformed conditions. We have used this reversible transformation model and investigated parasite control of host cell motile properties in the context of inflammatory signaling in Ma M. et al. [PLoS Pathog (2014) 10: e1004003]. We found that parasite infection promotes the production of the inflammatory cytokine TNFα in the host macrophage. We demonstrated that increased TNFα triggers motile and invasive properties by enhancing actin cytoskeleton remodeling and cell motility through the ser/thr kinase MAP4K4. We concluded that inflammatory conditions resulting in increased TNFα could facilitate cell dissemination by activating the actin cytoskeleton regulatory kinase MAP4K4. We discuss here the relevance of TNFα-MAP4K4 signaling for pathogen-driven cell dissemination and its potential impact on the induction of metastasis in human cancer.

PARASITE-ENFORCED ACQUISITION OF MOTILE PROPERTIES AND ITS ANALYSIS

The propagation of parasites inside their host or from one host to the next requires the acquisition of motile properties. In the case of intracellular parasitism, these properties can be triggered in the host cell, which allows the parasite to spread stealthily and protected from the immune system. This parasite-induced host cell dissemination and pathogen dispersion was referred to as Trojan horse strategy. Unlike the mythological horse, however, which had to be dragged into the city of Troy, parasitized host cells move autonomously. This is particularly striking in the case of dendritic cells, which within minutes of Toxoplasma or Neospora infection begin to migrate rapidly. Macrophages infected with Theileria annulata migrate in vitro and in vivo, whereby migration is parasite dependent because its elimination with the parasiticidal drug buparvaquone (BW720c) markedly alters the morphological and migratory proper-


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ties of the host cells. Host cell mobilization by the parasite requires an exchange between the parasite and host cell signaling but our understanding of parasite molecules controlling host cell functions remained marginal due to technical obstacles preventing the genetic manipulation of the parasite. However, the parasite can be experimentally eliminated by BW720c treatment and with it the source of promigratory signaling be disabled. This allows comparing motile behavior of parasite-infected with drug-cured cells of the same genetic background and characterizing host cell mechanisms needed for infected cell mobilization. Using such a comparative approach we have characterized T. annulata-dependent morphological and functional alterations in the host cell and investigated the underlying signaling pathways and molecular effectors.

![Graph](image)

**FIGURE 1:** (A) Control and MAP4K4-depleted MDA-MB231 breast cancer cells were analyzed in Boyden chamber transwell matrigel invasion assay. TNFα stimulation (25 ng/ml) significantly increases matrigel invasiveness of MDA-MB231 cells. If the potential proto-oncogenic ser/thr kinase MAP4K4 is depleted, invasive cell motility is largely blocked both under unstimulated as well as under TNFα stimulated conditions. (B) The downstream effector proteins of the ERM family are activated (phosphorylated) in response to TNFα stimulation (25 ng/ml) in MDA-MB231. Depletion of MAP4K4 blunts their activation. (C) Schematic overview of the proposed mechanistic linkage between TNFα stimulation and invasive cell motility. ECM: extracellular matrix.
Theileria JNK signaling is essential for survival and metastasis of serine/threonine kinase MAP4K4 to mediate inflammatory and metabolic processes. MAP4K4, a mechanistically relatively poorly understood molecule, has in recent years emerged as a key player in inflammatory and migratory processes including cancer progression. While trying to understand how these individual evidences may be connected, we began considering MAP4K4 as a potential hub diverting TNFα signals towards effectors that control F-actin dynamics and cell motility. We experimentally tested this possibility in *T. annulata*-infected cells and found that MAP4K4 indeed mediated the motile and invasive processes induced by TNFα. Rather unexpectedly, we also found that TNFα specifically activated the F-actin-plasma membrane cross-linker proteins of the ezrin, radixin, moesin (ERM) family and more generally increased F-actin assembly in cells, whereby both processes were impaired when MAP4K4 was depleted. From these studies we concluded that the increased motility and invasiveness we observed under conditions of chronically increased TNFα are the consequence of signal bifurcation at the level of MAP4K4, which ultimately couples inflammatory signaling to the regulation of actin dynamics and cell motility.

**DOES TNFα CAUSE INVASIVE MIGRATION OF HUMAN CANCER CELLS?**

Evidently, *T. annulata*-infected and transformed macrophages are different from metastatic cancer cells in several ways. Common to both, however, is the capability to disseminate and to breach tissue and extracellular matrix barriers. Our study revealed that invasive motility is driven by the permanent exposure of the infected cells to TNFα, which triggers and maintains F-actin assembly and turnover to drive cell movement. Could inflammation, in particular TNFα, also fuel dissemination of human cancer cells? The link between chronic inflammation, such as gastritis or hepatitis and cancer, has long been established and TNFα has emerged as a suspect of promoting cancer progression under these conditions. Moreover, a recent publication by Joan Massagué’s laboratory in breast cancer research showed that chemotherapeutics trigger the release of TNFα from stromal cells and that this TNFα release helps breast cancer cells to survive and metastasize. We therefore tested the possibility that breast cancer cells respond to TNFα with migration and invasion. Interestingly, analogous to *T. annulata* infected macrophages, MDA-MB231 breast cancer cells showed significantly increased motile and invasive properties when stimulated with TNFα (Fig. 1A). Importantly, these properties were blunted when MAP4K4 was depleted. Additionally TNFα stimulation of MDA-MB231 cells promoted the C-terminal phosphorylation of ERM proteins (Fig. 1B). Again, MAP4K4 was necessary for long term activation of ERM proteins in response to TNFα, combined suggesting that TNFα activation of cytoskeleton dynamics through MAP4K4 is functionally conserved.

Clearly, more in-depth analysis will be needed to fully clarify the functional significance of TNFα-MAP4K4 signaling for cancer cell progression (Fig. 1C). However, our study of host cell exploitation by an intracellular pathogen has
revealed an interesting link between inflammatory cytokine signaling and cell mobilization, which may also be relevant in cancer metastasis and immune cell mobilization under conditions of chronic inflammation such as rheumatoid arthritis.

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CONFLICT OF INTEREST
The authors have no conflict of interest to declare.

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