Review

CellPress

Theileria-transformed bovine leukocytes have cancer hallmarks

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The genus *Theileria* includes tick-transmitted apicomplexan parasites of ruminants with substantial economic impact in endemic countries. Some species, including *Theileria parva* and *Theileria annulata*, infect leukocytes where they induce phenotypes that are shared with some cancers, most notably immortalization, hyperproliferation, and dissemination. Despite considerable research into the affected host signaling pathways, the parasite proteins directly responsible for these host phenotypes remain unknown. In this review we outline current knowledge on the manipulation of host cells by transformation-inducing *Theileria*, and we propose that comparisons between cancer biology and host–*Theileria* interactions can reveal chemotherapeutic targets against *Theileria*-induced pathogenesis based on cancer treatment approaches.

Theileria-induced bovine immune cell transformation

Of the estimated 1.2–10 million species in the phylum Apicomplexa, only ~6000 have been described [1]. Almost all are intracellular parasites of vertebrate and invertebrate hosts, but the degree of diversity amongst these species is astounding. They have complex life cycles with diverse morphologies and are distributed over much of the globe. A member of the class Hematozoa, the genus *Theileria* includes tick-transmitted parasites of wild and domestic ruminants that cause a substantial economic burden (Box 1). A single species, *Theileria parva*, is responsible for >1 million cattle deaths per year in sub-Saharan Africa, at a cost of US$ >300 million [2]. The US Government 'Feed the Future' initiative (http://feedthefuture.gov/sites/default/files/resource/files/FTF_Guide.pdf) and the reformed Committee on World Food Security [3] have focused on reducing poverty and eliminating hunger from the world. In the wake of these renewed efforts, there has been a considerable boost in funding aimed to curb the impact of *T. parva* in sub-Saharan Africa.

Theileria parasites have several characteristics that make them unique among the known apicomplexa. During tick feeding, sporozoites are inoculated into the blood and infect white blood cells where they develop into schizonts [4]. Unlike many apicomplexans, *Theileria* resides in the host cytosol instead of inside a parasitophorous vacuole. During host cell mitosis, the schizonts bind to the host mitotic spindle, ensuring segregation into both daughter cells with great efficiency to maintain the infection rate [5].

With the fates of parasite and host cell closely intertwined, some *Theileria* species have evolved mechanisms to induce proliferation, immortalization, and dissemination of the host cell [6], arguably the phenotypes that most define cancer. In this review, when discussing *Theileria* parasites, we are only referring to transformation-inducing species in their cattle hosts (see Glossary). The most thoroughly studied are *T. parva*, which transforms bovine B and T lymphocytes, and *T. annulata*, which transforms macrophages, dendritic cells, and B cells [7]. Transformation in both cases is induced during the schizont stage (Figure 1). Both *T. parva* and *T. annulata* transform B cells, but whether or not the mechanism of host cell transformation is the same in both species remains to be established. Unfortunately, many relevant studies to date used only one or the other of these species; therefore, several comparative genomic studies have assumed the mechanism of host cell transformation to be the same [8–10]. Here we follow the same premise. *T. parva* and *T. annulata* are likely to have coevolved with different buffalo species (*Syncerus caffer* for *T. parva* and *Bubalus bubalis* for *T. annulata*) because these ruminant species appear to host the most diverse parasite populations and are not known to succumb to disease upon infection [11]. However, if left untreated, these parasites can kill susceptible cattle in less than 3 weeks, with a mortality that approaches 80% in some areas [12]. The conversion of *Theileria* schizont-infected cells into immortal cell lines depends on acquired characteristics that are remarkably similar to those exhibited by some cancerous cells, such as immune evasion and resistance to apoptosis. Each of these characteristics provides an opportunity for the development and use of cancer therapies for treating *Theileria* infections, and possibly a better understanding of the molecular interactions underlying these phenotypes. The goal of this review is to outline what is known about host cell manipulation by the transformation-inducing *Theileria* in the context of hallmarks of infection that are shared with many cancers [13].

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Keywords: Theileria; East Coast fever; cancer; cellular proliferation; hallmarks.

1471-4922/
 http://dx.doi.org/10.1016/j.pt.2015.04.001

Glossary

Autophagy: a process whereby cells degrade intracellular components to promote their own survival in response to cellular stress.
Classical dendritic cells: leukocytes that sense tissue injury, capture antigens, and present those antigens to T lymphocytes to induce immunity to foreign antigens and enforce tolerance to self-antigens.
Fas ligand: a protein that binds the Fas receptor and induces apoptosis upon binding, a mechanism used by cytotoxic T lymphocytes to induce apoptosis in target cells.
Hypoxia inducible factor 1α (HIF-1α): a transcription factor that regulates the cellular response to low oxygen conditions by activating the transcription of genes involved in energy metabolism, angiogenesis, and apoptosis.
IκB kinase complex (IKK complex): an enzyme complex consisting of three monogenic protein subunits (α, β, γ) that catalyzes the specific phosphorylation of the inhibitory IκB-α protein. IκB-α phosphorylation causes the dissociation of IκB-α from NF-κB, which then migrates to the nucleus and activates gene expression.
Interferon γ (IFN-γ): a cytokine produced mostly by T cells and natural killer cells that has antiproliferative, immunoregulatory, and proinflammatory activity during host defense.
Interleukin 2 (IL-2): a cytokine that is secreted by activated T cells and is important for lymphocyte proliferation, the clearance of self-reactive T cells, and the maintenance of regulatory T cells.
Leukocyte: white blood cells involved in immunity that circulate in the peripheral blood and consist of lymphocytes, monocytes, basophils, and neutrophils.
Lymphocyte: mononucleated leukocytes that include T cells, B cells, and natural killer (NK) cells.
Macrophage: a type of phagocytic leukocyte that differentiates from monocytes and plays crucial roles in host defense against pathogens, immune regulation, and wound healing.
Matrix metalloprotease 9 (MMP9): a secreted enzyme involved in the degradation of extracellular matrix that is crucial for homeostatic functions such as vascular development, cell migration, and wound repair.
MHC class I: a molecular complex found on nearly every nucleated cell that displays protein fragments, largely derived from the cytosol, to $CD8^+$ T cells and is crucial for the generation of antigen-specific adaptive immune responses and the inhibition of natural killer cell killing.
MHC class II: a family of molecules mostly found on professional, antigen-presenting cells such as dendritic cells, macrophages, and B cells that displays protein fragments derived primarily from extracellular proteins to $CD4^+$ T cells.
Nuclear factor κ-light chain enhancer of activated B cells (NF-κB): a conserved transcription factor protein complex canonically involved in the cellular production of cytokines and survival signals in response to stimuli such as stress and infection.
Phagocytosis: a process by which cells consume extracellular particles to form an internal vesicle containing those particles.
P53: an important tumor-suppressing transcription factor that regulates cellular responses to a myriad of cellular stressors including hyperproliferation, DNA damage, and telomere attrition.
Telomerase reverse transcriptase (*TERT*): a ribonucleoprotein enzyme that adds DNA repeats to the 3′ end of telomeric DNA at the ends of eukaryotic chromosomes.
Transformation: the modification of a eukaryotic cell to cause it to have some or all of the characteristics of a cancer cell (this is the definition used in this review and throughout the field of *Theileria* research).
Tumor growth factor β (TGF-β): a cytokine secreted by many cell types that regulates developmental programs and cell behavior by modulating cell proliferation, morphogenesis, differentiation, and tissue homeostasis and regeneration.
Tumor necrosis factor α (TNF-α): a cytokine produced by many immune and epithelial cell types that plays a central role in systemic inflammation, apoptosis, and immune system development.

Box 1. Outstanding questions and potential experimental approaches to address them

- Which parasite-derived molecules drive host proliferation? Screen parasite cDNA library for proliferation induction (e.g., carboxyfluorescein succinimidyl ester staining or cell counting).
- How do these parasites induce and maintain replicative immortality of their host cells? Quantify TERT activity in the presence or absence of NF-κB inhibitors.
- Which host genes regulate the metastasis of infected cells? Knockout known mammalian regulators of metastasis and quantify migration in a gel matrix.
- How do these parasites avoid autophagy despite host metabolic stress? Determine the effect of autophagy-inducing compounds on infection rates *in vitro* and *in vivo*.
- Which host molecules mediate contact-dependent proliferation of host cells? Screen mammalian and non-mammalian cells for an inability to support *Theileria* transformed bovine leukocyte growth.
- What is the effect of p53 sequestration on the *Theileria* surface during an infection? Alanine-scan p53 and screen for sequestration on the parasite surface.
- How do *Theileria* parasites maintain a carrier state in its bovine hosts? Determine if bTERT can be used as a vaccine in cattle.
- Do *Theileria* parasites induce genomic instability or mutations in their bovine hosts? Perform karyotyping and whole-genome sequencing of *Theileria*-transformed bovine leukocytes in comparison to their isogenic, uninfected controls.

Phenotypes *Theileria*-infected immune cells share with cancer cells

Despite many well-characterized differences among them, all cancer types share a defined set of phenotypes [13]. Several of these properties are also observed during *Theileria* infection.

Mechanisms of Theileria-induced proliferative signaling

Theileria-induced transformation leads to activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)-dependent proliferative signaling. NF-κB regulates a multitude of biological processes and is ubiquitously activated in hematological malignancies [14,15]. Pattern-recognition receptors can also activate NF-κB to induce *in vivo* antimicrobial programs that are crucial for innate and adaptive immunity [16], and many pathogens have the ability to suppress NF-κB signaling [17,18]. The IκB kinase (IKK) complex activates NF-κB, and *Theileria* schizonts have been shown to constitutively activate the IKK complex on their cell surface, possibly by *trans*-autophosphorylation [19]. Consequently, investigations into the mechanisms by which *Theileria* parasites manage to evade the immunostimulatory effects of NF-κB signaling provide an opportunity to discover novel therapeutics against *Theileria* infection.

Most healthy cells require a growth signal to undergo mitotic division as a mechanism to prevent inappropriate proliferation. For example, the multiplication of mature, naïve lymphocytes is largely regulated by antigen receptor stimulation as well as by a second, co-stimulatory signal [20]. While bovine leukocytes transformed by *T. parva* [21–24], but not by *T. annulata* [25,26], can produce interferon γ (IFN-γ) and interleukin 2 (IL-2), both produce and respond to tumor necrosis factor α (TNF-α) [27,28]. However, it has been suggested that some *Theileria*-transformed cell lines may grow independently of growth factors [29]. Cancer cells have been shown to achieve growth factor independence by (i) producing growth factor ligands themselves, (ii) sending stimulatory signals to nearby cells that provide growth factor ligands in return, (iii) becoming hyper-responsive to otherwise limiting levels of growth factor ligands, or (iv) constitutively activating downstream signaling pathways of a growth factor receptor [13]. A comparison of cell cycle-regulated genes between cancer and normal tissues revealed significant differences in proliferation programs and potential drug targets [14].