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Stress-sensing toll-like receptor as a driver of angiogenesis

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It is now experimentally established that angiogenesis and inflammation represent two prominent processes involved in normal physiological responses (e.g., wound healing) and pathological states (e.g., tumor growth and invasion). In addition, recruitment of myeloid cells during the inflammatory responses and neovascularization contribute to oxidative stress and production of free radicals or oxidized products. However, precisely how these processes are interrelated remains unclear. In a recent issue of *Nature*, West et al. provide new insights into the linkage of inflammation and angiogenesis by demonstrating that oxidized phospholipids generated from inflammation-associated oxidative stress engage toll-like receptor 2 (TLR2) on endothelial cells (ECs), thereby promoting angiogenesis (West et al., 2010).

The essential roles of the TLR family of receptors in recognizing foreign pathogens and initiating the cascade of host defense responses through innate as well as adaptive immunity have been established (Palm and Medzhitov, 2009). Upon ligand binding to TLR, the adaptor molecule MyD88 is recruited to the TLR complex, which consequently promotes association with and activation of a series of signaling adaptors, kinases and transcription factors, such as NF- κ B, a master transcription factor that controls the expression of various inflammatory cytokines and chemokines. Additional toll/interleukin-1-receptor (TIR)-domain-containing adaptor proteins have also been identified, including TIR-domain-containing adaptor protein inducing interferon- β (TRIF). The TLR-TRIF signaling pathway mediates MyD88-independent induction of interferon- β . In addition to sensing pathogen-associated molecular pattern molecules (PAMPs) present in foreign pathogens, recent studies have documented that TLRs also appear to be activated by endogenous, damage-associated molecular pattern molecules (DAMPs) that are released during stress conditions or following injury (Seong and Matzinger, 2004). Although the view of endogenous self-molecules as TLR ligands is controversial due to potential contamination issues, mounting evidence from *in vivo* studies supports the concept of an endogenous ligand for TLRs (Schaefer, 2005 #1408). DAMPs-triggered TLR activation leads to subsequent inflammatory responses that may signal 'danger' to the host immune response. The coordinated events underlying inflammation are important to wound healing and tissue repair. However, the consequences of inflammation persist in many pathologic conditions, such as cancer or other inflammatory diseases. Indeed, West et al. have identified carboxyalkylpyrrole protein adducts (CAPs), such as ω -(2-carboxyethyl) pyrrole (CEP) derived from lipid oxidation, as a new class of DAMP that acts through TLR2. More importantly, CEP-TLR2 interaction facilitates EC-mediated vascularization during tissue injury and repair. Furthermore, persistent accumulation of CEP and its analogues in tissues and excessive engagement of TLR2 may also play a critical role in tumor angiogenesis during carcinogenesis.

The authors made initial observations that CEP levels correlated with inflammation-induced vascularization during wound healing, as indicated by transient elevation of CEP expression in injured tissues. Furthermore, CEP levels profoundly increased in highly vascularized murine melanoma and the majority of CEP was shown to be present in tissue macrophages, implicating CEP in inflammation and angiogenesis in the physiologic and self-limiting wound healing process as well as in pathologic, uncontrolled tumor growth. Subsequent

studies in both human and mouse endothelial cells lead to an intriguing finding that CEP promotes angiogenic responses independent of vascular endothelial growth factor (VEGF) receptor-mediated signaling. Given the fact that other adducts from the same family of CAPs also stimulate vascularization, the authors hypothesized that a particular molecular pattern, rather than a specific chemical moiety, is involved in the promotion of angiogenesis via interaction with pattern recognition receptors, such as TLRs.

West and colleagues demonstrate that antibody-mediated blockade of TLR2, but not TLR4, inhibited CEP-stimulated tube formation and EC migration, suggesting that CEP is a ligand of and recognized by TLR2. Further support came from the finding that TLR2-deficient ECs failed to respond to CEP and the TLR2 synthetic ligand Pam3CSK4. Additional studies showed that administration of exogenous CEP effectively promoted vascularization in a TLR2-dependent manner using two tissue injury systems, including a hindlimb ischaemia model and a wound closure and vascularization model. Bone marrow chimera experiments showed that TLR2 on non-haematopoietic cells or radiation resistant cells are primarily involved in CEP-stimulated vascularization during wound healing and murine melanoma angiogenesis.

Neutralizing antibodies for CEP was used to define the contribution of endogenously generated CEP to the process of vascularization in wound healing and tumor growth. Intravenous delivery of CEP-blocking antibodies significantly impaired vascularization and tissue repair. Administration of these antibodies also similarly diminished vascularization of mouse melanoma as well as inhibiting tumor progression. Consequently, these studies not only establish a new property of TLR2 as a sensor of molecular patterns resulting from oxidative stress-induced modification of lipid proteins, but also underscore the importance of DAMP-TLR interactions in both physiological and pathological circumstances.

Although cancer is considered to be fundamentally a genetic disease, the reciprocal interactions between genetically altered cancer cells and normal host cells, including inflammatory cells, endothelial cells and fibroblasts in the tumor microenvironment contribute profoundly to the development of an invasive cancer (Grivennikov et al., 2010). While the concept that inflammation may potentiate tumor development and progression is gaining momentum in the scientific community, the precise molecular mediators that regulate this complex response remain largely unexplored. The authors provide the first evidence demonstrating that CEP generated during inflammatory processes-induced oxidative stress drives melanoma angiogenesis through pattern recognition TLR2. Another recent study showed that the extracellular matrix proteoglycan versican can activate TLR2 on macrophages, resulting in increased TNF- α production and enhanced lung cancer metastasis (Kim et al., 2009). It appears that persistent release or production of endogenous TLR2 ligands, exemplified by CEP and versican as well as other DAMPs (e.g., HMGB1, hyaluronan), can act on different host cells, leading to establishment of a chronic inflammatory state that favors tumor progression and invasion. More importantly, given that CEP drives angiogenesis through TLR2-MyD88 pathways independent of VEGF receptor signaling, targeting CEP may offer a novel therapeutic strategy for treatment of cancers that are resistant to classical anti-VEGF therapy.

It is becoming clear that TLRs not only play a fundamental role in the recognition of pathogens and in the initiation of immune responses, but also are active participants in normal physiological processes as well as non-infectious disease-associated pathogenesis, which are strongly supported by the findings of West et al. While the study provides a novel mechanism for inflammation-associated oxidative stress and angiogenesis, it also raises several new questions that need to be addressed. It remains unclear precisely how TLR2-mediated signaling or CEP-triggered molecular events in ECs are involved in the functional

regulation of ECs, which lead to enhanced vascularization in injured tissue and melanoma. Although the investigators did not provide evidence in this study, CEP-TLR2-induced inflammatory responses in ECs are expected to be essential for the enhanced vascularization given the established role of TLR2 in innate immunity. It would also be interesting to explore whether CEP may be one of the previously unrecognized pathogenic factors involved in other inflammatory diseases, such as atherosclerosis. Furthermore, it has been shown that CEP-TLR2 interactions on ECs appear to facilitate melanoma angiogenesis as well as tumor growth, whereas versican-TLR2 interactions on myeloid cells (e.g., macrophage) only influence lung tumor metastasis, but not primary subcutaneous tumor growth (Kim et al., 2009). Multiple DAMPs in the tumor microenvironment may engage the same or different TLRs or even other pattern recognition receptors. How these interactions and resultant complex responses regulate or contribute to tumor progression, escape and invasion remain to be elucidated. Based on the observations of CEP-promoted melanoma angiogenesis, it is clearly worth investigating the action of CEP in tumor metastasis using different model systems, including clinically relevant transgenic melanoma mice and spontaneous metastatic lung tumor models. Defining the precise contribution of CEP in tumor progression and invasion would establish the rationale for developing CEP-targeted novel therapeutics for clinical translation.

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Figure 1.