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NPAT mutations in Hodgkin lymphoma

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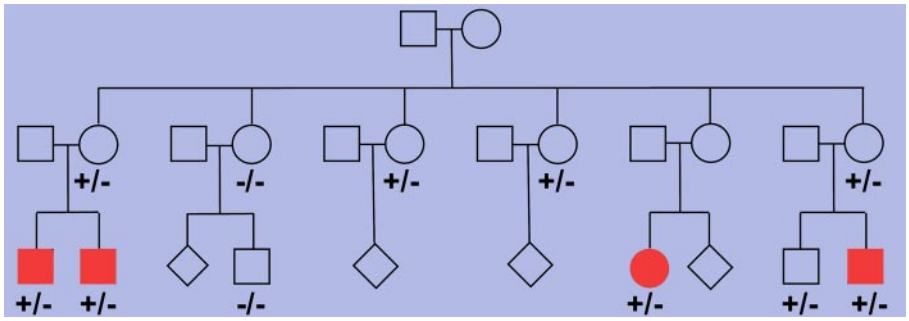
The pathogenesis of both classic and nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) is still enigmatic. In this issue of *Blood*, Saarinen and colleagues report the identification of germ line mutations in the *NPAT* gene as a candidate risk factor for Hodgkin lymphoma.[1]



Pedigree of a Finnish family with multiple members affected by nodular lymphocyte predominant Hodgkin lymphoma. Multiple family members were tested for the presence of the truncating *NPAT* mutation and each of 4 cousins affected by the lymphoma showed this mutation (depicted as filled symbols). −/− indicates wild-type for the 2 base pair deletion in the *NPAT* gene and +/− indicates presence of the deletion on 1 allele. Where no genotype is indicated, no DNA was available for analysis. The pedigree has been modified for confidentiality.

Hodgkin lymphoma is one of the most frequent lymphomas in the Western world. Based on differences in tumor cell morphology and phenotype and the histologic picture, this lymphoma entity is subdivided into a classic form and NLPHL, the former accounting for about 95% of cases. We know relatively little about the genetic lesions causing Hodgkin lymphoma, partly because of the rarity of the tumor cells in affected lymph nodes, which hampers the molecular analysis of these cells for somatic alterations. Saarinen and colleagues combined 2 modern genetic methods, single nucleotide polymorphism chips and whole exome sequencing, and applied them to a unique Finnish family with 4 cousins all presenting from 22-26 years of age with NLPHL.[1] A germ line mutation in the *NPAT* (nuclear protein, ataxia-telangiectasia locus) gene was found in all 4 patients (see figure). The mutation was a 2 base pair deletion causing a frame shift and in consequnce a truncation of the protein. Screening of many more Hodgkin lymphoma patients and healthy controls did not reveal additional instances of this mutation but, importantly, a replacement mutation in codon 724 of the gene was found in other NLPHL and classic Hodgkin lymphoma patients at significantly higher frequency than in healthy controls. This identifies *NPAT* germ line mutations as the first candidate genetic risk factor for NLPHL.

Although it is intriguing that 2 different types of germ line mutations affecting the NPAT protein sequence were found in Hodgkin lymphoma patients, the functional consequences of these mutations remain presently unclear, and deserves future study. NPAT has been reported to be involved in the regulation of the cell cycle, and it is presumably also involved in the regulation of the *ATM* tumor suppressor gene, which is located directly adjacent to *NPAT*. Thus, a pathogenetic role of the mutations can be envisioned. However, it is not clear whether the mutated *NPAT* gene acts as an oncogene or a tumor suppressor gene. If one assumes that the mutations cause a loss of function, it would be important to clarify whether the second allele of the gene is somatically mutated in the lymphocyte predominant (LP) or Hodgkin and Reed-Sternberg cells with germ line mutations on 1 allele. Alternatively, the mutations may have a dominant negative effect (eg, if the protein functions as a dimer or multimer), so that the germ line mutation on 1 allele is sufficient to cause a loss of function. Finally, NPAT may show haploinsufficiency in HL, that is, the amount of remaining wild-type protein encoded from the second allele is not sufficient to sustain normal NPAT function. Cells from patients with *NPAT* mutations indeed showed reduced expression levels of the gene.[1]

NPAT germ line mutations were found in NLPHL as well as in classic HL cases, suggesting that such mutations may contribute to the pathogenesis of both subtypes of Hodgkin lymphoma. This is a remarkable finding, because although classic and NLPHL are considered 2 forms of 1 disease, there is presently little indication for common genetic lesions or other shared transforming events in the tumor cells of these lymphomas.[2] For example, even though strong constitutive activity of the NF-κB transcription factor signaling pathway is a hallmark of the lymphoma cells in both classic and NLPHL,[2,3] the mechanisms for this activation appear to be very distinct: somatic inactivating mutations in the negative NF-κB regulators TNFAIP3 and NFKBIA are frequent in the Hodgkin and Reed-Sternberg tumor cells in classic Hodgkin lymphoma, but they do not play a significant role in the LP tumor cells of NLPHL.[2,4] Moreover, infection of the tumor cells by Epstein-Barr virus contributes to NF-κB activation in classic Hodgkin lymphoma, but is not seen in NLPHL.[2] Somatic mutations in the *SOCS1* gene, encoding for a negative regulator of the JAK/STAT signaling pathway, were, before the study by Saarinen et al, the only known genetic lesion detected in both types of Hodgkin lymphoma.[5,6]

Besides the genetic lesions in the *SOCS1* gene, we know hardly anything about the transforming events in NLPHL. The only other recognized recurrent genetic lesion in these cells are translocations involving the proto-oncogene *BCL6*.[7] BCL6 is a transcription factor that regulates the germinal center B-cell differentiation program. Translocations of *BCL6* may thus contribute to NLPHL pathogenesis by freezing the LP tumor cells in the highly proliferative germinal center B-cell differentiation stage.

As Hodgkin lymphoma shows an increased familiar association,[8] there is the suspicion that germ line mutations or polymorphisms may represent predisposing factors. With modern genome-wide association studies (GWAS) and next-generation sequencing methods, we now have better tools to identify such predisposing

factors. For classic Hodgkin lymphoma, a recent GWAS study identified risk loci at the *REL*, *PVT1*, and *GATA3* loci,[9] and confirmed an association of Hodgkin lymphoma risk with the HLA region.[10] Through the work by Saarinen et al, a first candidate predisposing factor for NLPHL has been identified, and a further factor for classic Hodgkin lymphoma. This finding has to be validated in an independent study, but it demonstrates the power of the genome-wide methods and advances our understanding of Hodgkin lymphoma pathogenesis.

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Comment on Elks et al, page 712

HIFs: a-cute answer to inflammation?

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In this issue of *Blood,* Elks et al describe a novel role for activated hypoxia inducible factor (HIF)–1α in sustaining inflammation by delaying neutrophilic retrograde emigration and preventing neutrophil apoptosis through the inhibition of prolyl hydroxylase (PHD) activity. This property is a novel function for HIFs, the master regulators of our body's response to hypoxia.

HIFs are hypoxia-driven transcription factors that are transcribed and translated constitutively. In oxygen-rich environments, the half-life of HIF-α is limited by prolyl-hydroxylation, ubiquitination, and proteosomal degradation of the translated protein. At normoxia, prolyl hydroxylases (PHDs) hydroxylate the HIF-α subunits on critical proline residues and the hydroxylated proteins are recognized by ubiquitin ligases like von Hippel-Lindau proteins, targeting the HIF-α subunits for degradation. In the absence of oxygen, hydroxylation of the HIF-α protein is blocked, resulting in HIF-α pairing with HIF-β (ARNT), translocating to the nucleus and binding to hypoxic response elements (HREs) to induce gene transcription. The article by Elks et al[1] targets HIF-1α, the master regulator of vascular endothelial growth factor (VEGF), as the focused isoform. HIF-1α activation is not only associated with tissue angiogenesis, an adaptive response to tissue hypoxia, but also vascular development, metabolism, inflammation, and cellular processes such as differentiation, survival, and autophagy. For example, HIF-1α is directly implicated in epithelial-to-mesenchymal transition (EMT), an important process in cancer and in repair/remodeling disease and a putative mechanism to produce collagen-producing cells in an affected compartment. HIF-1α expression is critical developmentally, as deletion of this gene results in fetal loss from the lack of vasculature in the animal. Interestingly, the absence of another HIF-α isoform, HIF-2α, does not mirror the developmental problems of HIF-1α, suggesting nonoverlapping roles for HIF-1α and HIF-2α in health and disease.

The first published observation connecting inflammation and the HIF pathway was by Hellwig-Bürgel et al *Blood* in 1999, illustrating that IL-1β and TNF-α augment cellular DNA binding of HIF-1α at normal oxygen in human hepatoma cells. Moreover, combining IL-1β and TNF-α with hypoxia to stimulate cells creates a synergistic effect on DNA binding and cellular activation. The initial model proposed that HIF-1α directly modulates gene expression during inflammation.[2] By incorporating a zebrafish model, Elks and colleagues are the first to report that HIF-1α directs inflammation by interrupting the migratory behavior of neutrophils during the resolution phase of inflammation in a whole body organism. By generating dominant-active and dominant-negative variants of the 2 zebrafish homologues of HIF-1α (HIF-1αa and HIF-1αb), they show that temporal resolution of neutrophil-mediated inflammation and neutrophil survival is dependent on prolyl hydroxylation. Their report in this issue of *Blood* demonstrates further detail about the complex activities of HIF-1α in orchestrating the acute inflammatory response. Further, they show neutrophil-specific expression of a dominant-active isoform HIF-1αb is sufficient to modulate the resolution of neutrophilic inflammation while dominant-negative HIF-1αb abrogates the effects of the hypoxia-mimic, DMOG, a pan-prolyl hydroxylase inhibitor.

Why is the HIF pathway so important in acute and chronic inflammatory diseases? Several well-known HIF-1α–regulatable genes expressed during hypoxia include the potent angiogenic factor, *VEGF,* which induces tissue remodeling, enhances vascular permeability, and enhances TH_2-mediated sensitization and lung inflammation[3]; *erythropoietin*, a glycoprotein that stimulates red blood cell production from the bone marrow; *Glut-1*, or glucose transporter 1, which transports glucose across endothelial membranes during episodes of increased glycolysis such as hypoxia; several *MMPs* that continuously remodel inflamed tissue releasing matrix-bound proinflammatory proteins; and *CXCR4* and other