NFkB Activation in Primary Mediastinal Large B-cell Lymphoma:
Nuclear Localization of c-REL and Coordinate Upregulation of NFkB Target Genes


Primary Mediastinal Large B-Cell Lymphoma (MLBCL)

Typical localization, age distribution and morphology

Important differences from Diffuse Large B-Cell Lymphoma (DLBCL), and shared features with classic Hodgkin's Lymphoma (cHL):

- Downregulation of BCR signaling cascade
- Upregulation of cytokine pathways, TNF family members, and ECM elements
NFkB Activation: a Potential Survival Pathway in MLBCL?

Constitutive NFkB activation known to inhibit apoptosis in Hodgkin Reed-Sternberg cells

Upregulation of specific NFkB target genes in the MLBCL transcriptional signature

Predominantly nuclear localization of the c-REL NFkB subunit in MLBCL indicates activation of pathway in our previous pilot study

DLBCL Subsets Defined by Transcriptional Profiles

…based on similarity with normal B-cell developmental stages (Wright et al. 2002):
- **GCB** (Germinal center B-cell)
- **ABC** (activated B-cell)
- “other” (not assigned)

…based on consensus clustering in the space of genome-wide expression data (Monti et al. 2004):
- **OXP** (Oxidative phosphorylation)
- **BCR** (B-cell receptor / proliferation)
- **HRS** (Host Response)
Immunohistochemical detection of c-REL subcellular localization in tumor samples

Translocation of active NFkB dimer to nucleus

(modified from: Karin M, Nat Rev Cancer 2; 301, 2002)

Subcellular localization of c-REL

Immunohistochemical detection of c-REL confirms the predominantly nuclear localization in MLBCL with a broadly available immunoperoxidase method.
Assessment of NFkB DNA binding activity by an colorimetric assay

Detection of NFkB DNA binding (modified from: Karin M, Nat Rev Cancer 2; 301, 2002)

Level of NFkB Activity in the Karpas 1106 (MLBCL-derived) Cell Line

NFkB activity in Karpas 1106 cells compares to those of HL cells and LY10, a NFkB dependent DLBCL cell line
NFkB Inhibition by Retroviral Transduction of Mutant IkB (SR-IkB)

Inhibition of NFkB activity by super-repressor SR-IkB

Detection of NFkB DNA binding

(modified from: Karin M, Nat Rev Cancer 2; 301, 2002)

SR-IkB Expression Results in Efficient Inhibition of NFkB Activity and Increase of Apoptosis in Karpas 1106 Cells

Decrease of NFkB DNA binding activity

Increase of Apoptosis (Annexin V binding)
NFkB Inhibition in Karpas 1106 (MLBCL) Results in Decrease of Proliferating Cells

![Graph showing MTS (Absorbance) over time (hours)]

NFkB Target Gene Sets


2. Known NFkB target genes differentially expressed at B-cell developmental stages / DLBCL cell lines with “ABC-like” features (Shaffer et al. Immunity 2001; Davis et al. J Exp Med 2001)

3. NFkB target genes downregulated after siRNA silencing of REL-A (p65) in TNFα stimulated HeLa cells (Zhou et al. Oncogene, 2003)
Increased Expression of NFkB Target Genes in MLBCL and ABC-like DLBCL

c-REL amplification and c-REL transcript abundance in LBCL subtypes

Amplification of the c-REL locus is associated with high c-REL transcript abundance, but not with DLBCL subtypes that are characterized by a NFkB activation signature.

Increased Expression of NFkB Target Genes in “Host Response” (HRS) DLBCL
“NFkB – signatures” in LBCL subtypes

Conclusions

1. NFkB signaling is consistently activated in MLBCL. This does not require amplification of the c-REL locus.

2. The consequences of NFkB activation are different in individual DLBCL subsets and may be dependent on specific developmental features and the tumor microenvironment.

3. Amplification of the c-REL locus is not closely associated with prominent NFkB activation signatures in DLBCL subsets.
## Participants

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