

NFkB Activation in Primary Mediastinal Large B-cell Lymphoma:

Nuclear Localization of c-REL and Coordinate Upregulation of NFkB Target Genes

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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?

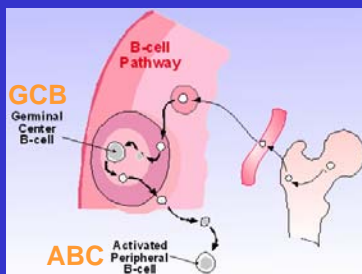
Constitutive NFkB activation known to inhibit apoptosis in Hodgkin Reed-Sternberg cells

Upregulation of specific NFkB target genes in the MLBCL transcriptional signature

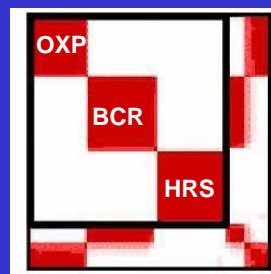
Predominantly nuclear localization of the c-REL NFkB subunit in MLBCL indicates activation of pathway in our previous pilot study

DLBCL Subsets Defined by Transcriptional Profiles

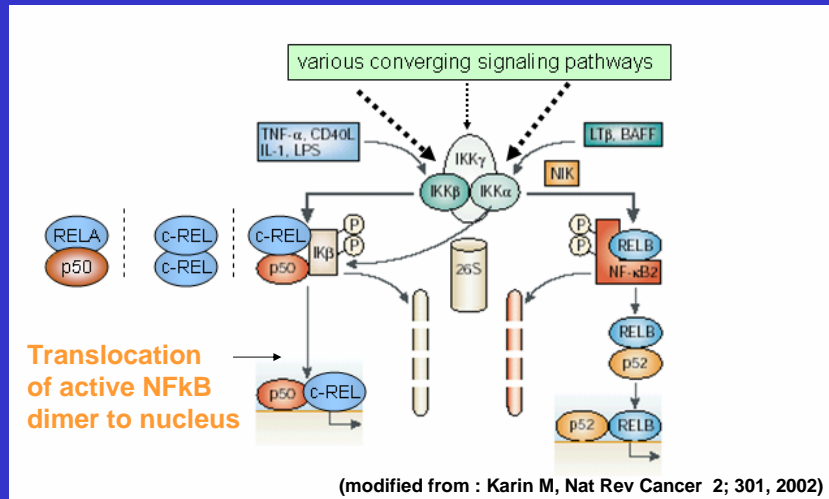
...based on similarity with normal B-cell developmental stages (Wright et al. 2002):
GCB (Germinal center B-cell)
ABC (activated B-cell)
"other" (not assigned)



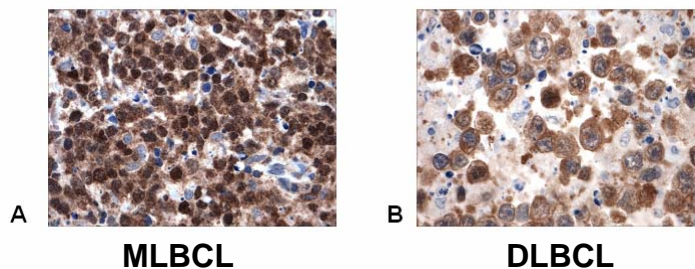
...based on consensus clustering in the space of genome-wide expression data (Monti et al. 2004):
OXF (Oxidative phosphorylation)
BCR (B-cell receptor / proliferation)
HRS (Host Response)



Immunohistochemical detection of c-REL subcellular localization in tumor samples

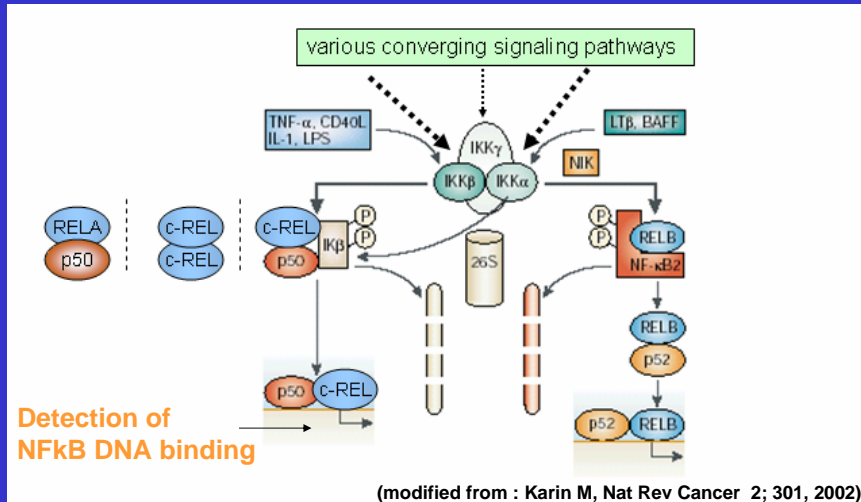


Subcellular localization of c-REL

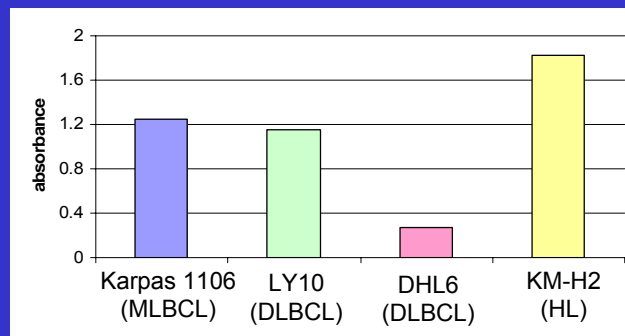


Immunohistochemical detection of c-REL confirms the predominantly nuclear localization in MLBCL with a broadly available immunoperoxidase method.

Assessment of NFkB DNA binding activity by an colorimetric assay

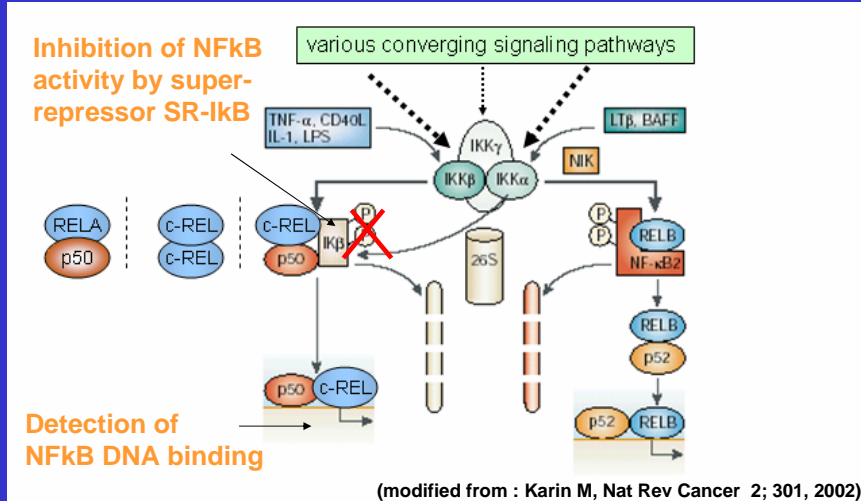


Level of NFkB Activity in the Karpas 1106 (MLBCL-derived) Cell Line

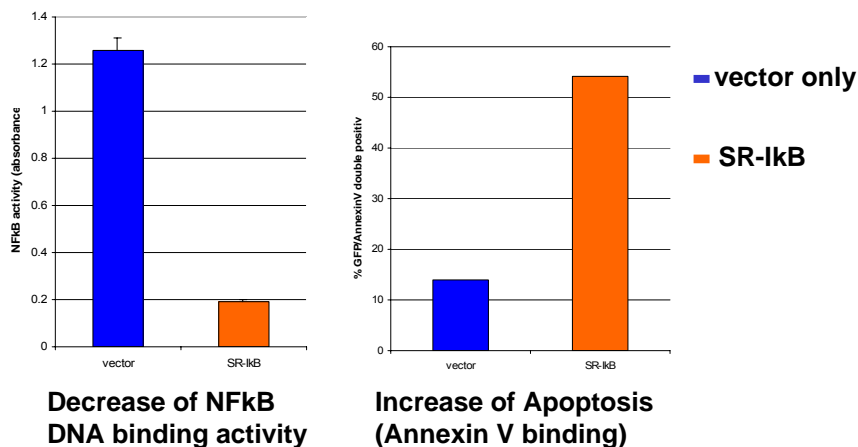


NFkB activity in Karpas 1106 cells compares to those of of HL cells and LY10, a NFkB dependent DLBCL cell line

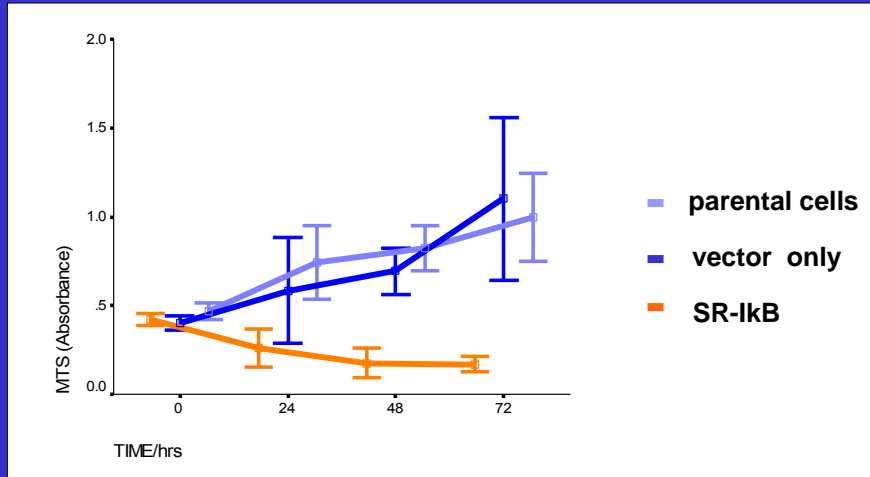
NFkB Inhibition by Retroviral Transduction of Mutant Ikb (SR-IkB)



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



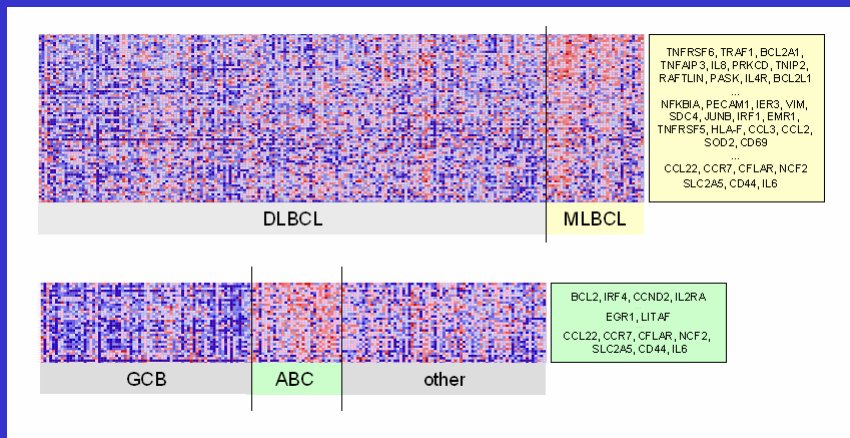
NFkB Inhibition in Karpas 1106 (MLBCL) Results in Decrease of Proliferating Cells



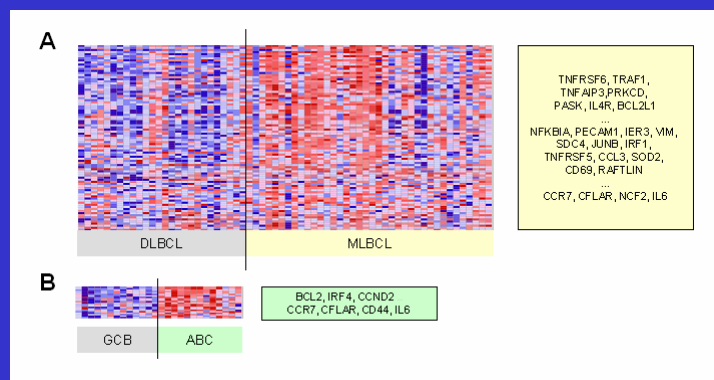
NFkB Target Gene Sets

- (1) NFkB target genes downregulated in HL cells following introduction of NFkB super-repressor (Hinz et al. J Exp Med, 2002)
- (2) Known NFkB target genes differentially expressed at B-cell developmental stages / DLBCL cell lines with "ABC-like" fetures (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 TNF α stimulated HeLa cells (Zhou et al. Oncogene, 2003)

Increased Expression of NFkB Target Genes in MLBCL and ABC-like DLBCL

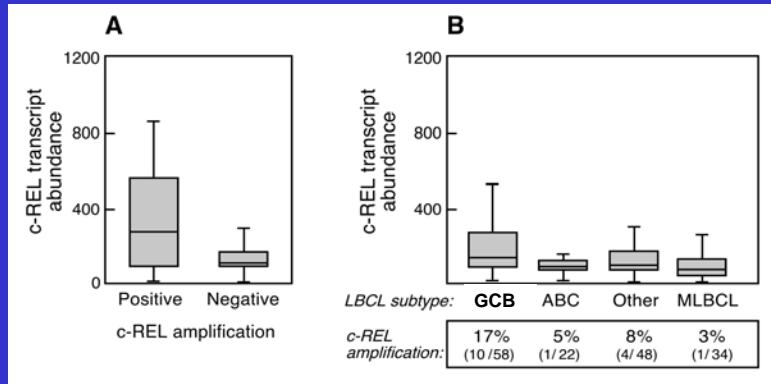


Increased Expression of NFkB Target Genes in MLBCL and ABC-like DLBCL: Confirmatory Series



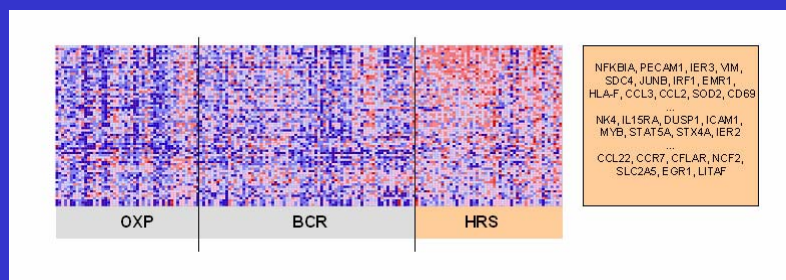
(Rosenwald A et al. J Exp Med, 2003; data available at <http://Immpp.nih.gov>)

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

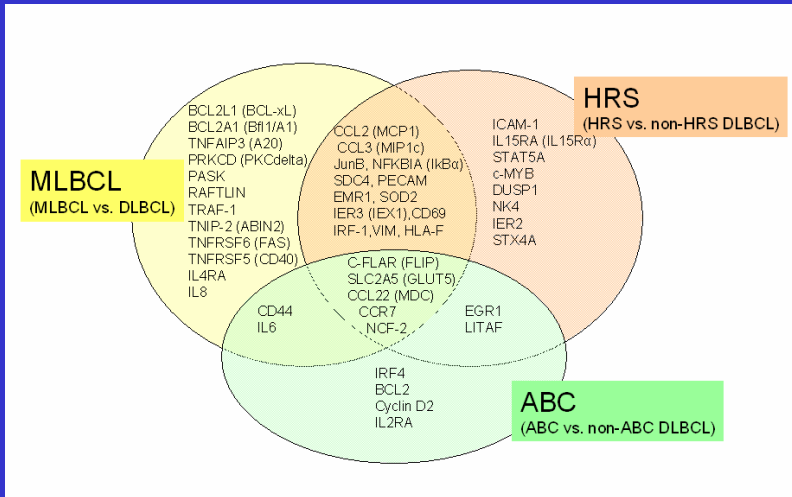


Amplification of the c-REL locus is associated with high c-REL transcript abundance, but not with DLBCL subtypes that are characterized by a NF κ B activation signature

Increased Expression of NF κ B 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

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