Supplementary data on the differential expression and regulation of p38 MAPK isoforms by IL-4 in lymphoma-derived cell lines

Supplementary Fig. 1 (a) Expression of α, β, γ, δ isoforms of p38 MAPK mRNA in cell lines derived from DLBCLs compared to that seen in purified germinal center B cells. (b) Expression of α, β, γ, δ isoforms of p38 MAPK mRNA normalized to that seen in purified germinal center B cells (GCB). Each data point represents the mean of triplicate quantitative fluorescence PCR assays.
Supplementary Fig. 2 Effect of IL-4 (5ng/mL) on expression of α, β, γ, δ isoforms of p38 MAPK mRNA in DLBCL-derived cell lines. Each data point represents the mean of triplicate quantitative fluorescence PCR assays and is normalized to that seen in untreated control cells.
Results

Expression of isoforms of p38 MAPK mRNA DLBCL-derived cell lines

The 4 isoforms of p38 MAPK, α, β, γ and δ are thought to represent related but distinct substrates specificities and thus biologic actions. Furthermore, the distribution of p38 MAPK isoforms has been shown to be very tissue specific. To determine which isoforms are expressed highly in B-lymphocytes, we performed quantitative real-time RT-PCR for the expression of p38 MAPK α, β, γ and δ isoforms. Transcripts of all four isoforms of p38 MAPK were detected in the 4 DLBCL derived cell lines (OCI-Ly-10, OCI-Ly-1, Sudhl-4, and Sudhl-6) that initially showed significant expression of p38 MAPK. The most predominant isoform expressed in all the cell lines was p38γ, followed by p38β (Supplementary Fig. 1). In Sudhl-6 cells, p38δ was the least expressed, while in the other three cell lines, p38α was the lowest. As compared to normal germinal center B cells, OCI-Ly-1, OCI-Ly-10, and Sudhl-4 over-expressed p38δ, but showed lower expression of p38α, p38β, and p38γ. Sudhl-4 demonstrated relatively higher levels of expression of the p38β isoform (Supplementary Fig. 1). Sudhl-6 showed the least overall expression of isoforms of p38 MAPK mRNA as compared to the other three cell lines (Supplementary Fig. 1), and indeed, the expression of all four isoforms of p38 MAPK mRNA in Sudhl-6 was reduced to 15% to 58% of that in normal germinal center B cells.

Effects of IL-4 on expression of isoforms of p38 MAPK gene

To determine the effect of IL-4 on expression of different isoforms of the p38 MAPK gene, we performed quantitative real-time RT-PCR on cell lines exposed to 5 ng/mL of IL-4 for a 24 hour time period. IL-4 increased the expression of all isoforms of p38
MAPK mRNA in all cell lines except in Sudhl-4 (Supplementary Fig. 2), with the increase ranging from 1.91, and 2.66 fold of untreated cells. This was cell-type specific and most significant changes were observed in OCI-Ly-1, and Sudhl-6 cell lines, with little effect seen in OCI-Ly-10 and Sudhl-4 cell lines. The δ isoform was down-regulated by IL-4 in the Sudhl-4 cell line whereas in other cell lines it was up-regulated by IL-4. IL-4, however, displayed no significant induction of expression of α isoform in OCI-Ly-1 and Sudhl-6 cells. IL-4 did not show significant effects on expression of all four isoforms of p38 MAPK mRNA in OCI-Ly-10 and Sudhl-4 (Supplementary Fig. 2).

**Discussion**

The p38 MAPK family is composed of four members, namely p38α, p38β, p38γ, and p38δ [2, 13-18]. In addition to high homology, the family is defined by a TGY motif within kinase subdomain seven. Dual phosphorylation of this domain in the four isoforms is required for p38 activation [41, 42]. Although these protein kinases are related, they are significantly distinct in substrate specificity [20], inhibition mechanisms [19], activation by extracellular stimuli [21, 22], and biological actions [23, 24]. Tissue distribution of different isoforms of p38 MAPK mRNA has been studied [2, 13-18], however there is no information on expression of these isoforms in B-cells or B-cell lymphoma cells. Our data show that both reactive germinal center B cells and cells derived from DLBCL express all 4 isoforms of p38 MAPK at the RNA level. The most predominant isoform of p38 MAPK expressed in cell lines derived from DLBCL was p38γ, followed by p38β while the expression of p38α and p38δ was minimal.
The expression profile of p38 MAPK isoforms in DLBCL-derived cells differed from other non B-cell lines or non-lymphomatous tissues that have been studied. For instance, previous studies showed that the tissue distribution of p38γ is very limited as compared to p38α, β, and δ, and is highly expressed in skeletal muscle, but is only expressed at low levels in other tissues [2, 13-18]. Among inflammatory cell lineages, p38α is the dominant form of p38 MAPK in monocytes, while the expression of p38δ is low and there is no detection of p38β isoform. Macrophages express abundant levels of both p38α and p38δ, but show minimal expression of p38β. The expression of both p38α and p38δ are seen in neutrophils, CD4+ T-cells and endothelial cells. Notably, unlike DLBCL-derived cell lines, p38γ is not detected in any other inflammatory cell types [43]. Differential expression of p38 MAPK isoforms in DLBCLs suggests that distinct isoforms of p38 MAPK may play different roles in the pathogenesis of B cell lymphomas.

Furthermore, IL-4 stimulation resulted in differential regulation of p38 MAPK isoforms in a cell-type specific manner. In OCI-Ly-1 and Sudhl-6 cell lines, all isoforms were up-regulated by IL-4 while in OCI-Ly-10 and Sudhl-4 cell lines; there was no significant expression of any of the isoforms. These data further highlight the complexity of regulation of p38 MAPK isoform expression by which IL-4 may play a role in regulating p38 MAPK pathway.