Abstract #28 | Poster

IGH enhancer RNAs - new players in B-cell lymphoma

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Chromosomal translocations in non-Hodgkin lymphoma (NHL) result in activation of oncogenes by placing them under the regulation of immunoglobulin heavy chain (IGH) super-enhancers. Aberrant expression of translocated oncogenes induced by enhancer activity can contribute to lymphomagenesis. The role of the IGH enhancers in normal B-cell development is well established, but knowledge regarding the precise mechanisms of their involvement in control of the translocated oncogenes is limited. We performed a tiling CRISPR/Cas9 interference screen in three NHL cell lines and identified three DNA regions crucial for NHL cell growth (Figure 1). With chromatin-enriched RNA sequencing we showed transcription from the core enhancer regions and subsequently validated expression of the enhancer RNAs (eRNA) in a panel of NHL cell lines and tissue samples. Inhibition of the essential IGH enhancer regions decreased expression of eRNA and translocated oncogenes in several NHL cell lines.

We further set out to determine whether the eRNA transcripts themselves are essential. Targeting at one of the eRNAs with Cas13d led to a significant decrease in growth of three Burkitt lymphoma cell lines (Figure 2). Moreover, it resulted in decreased IgM surface expression (Figure 3) and reduced MYC protein levels. This suggests that the IGH eRNA is essential for lympohoma cells survival and indicates it as a potential therapeutic target.

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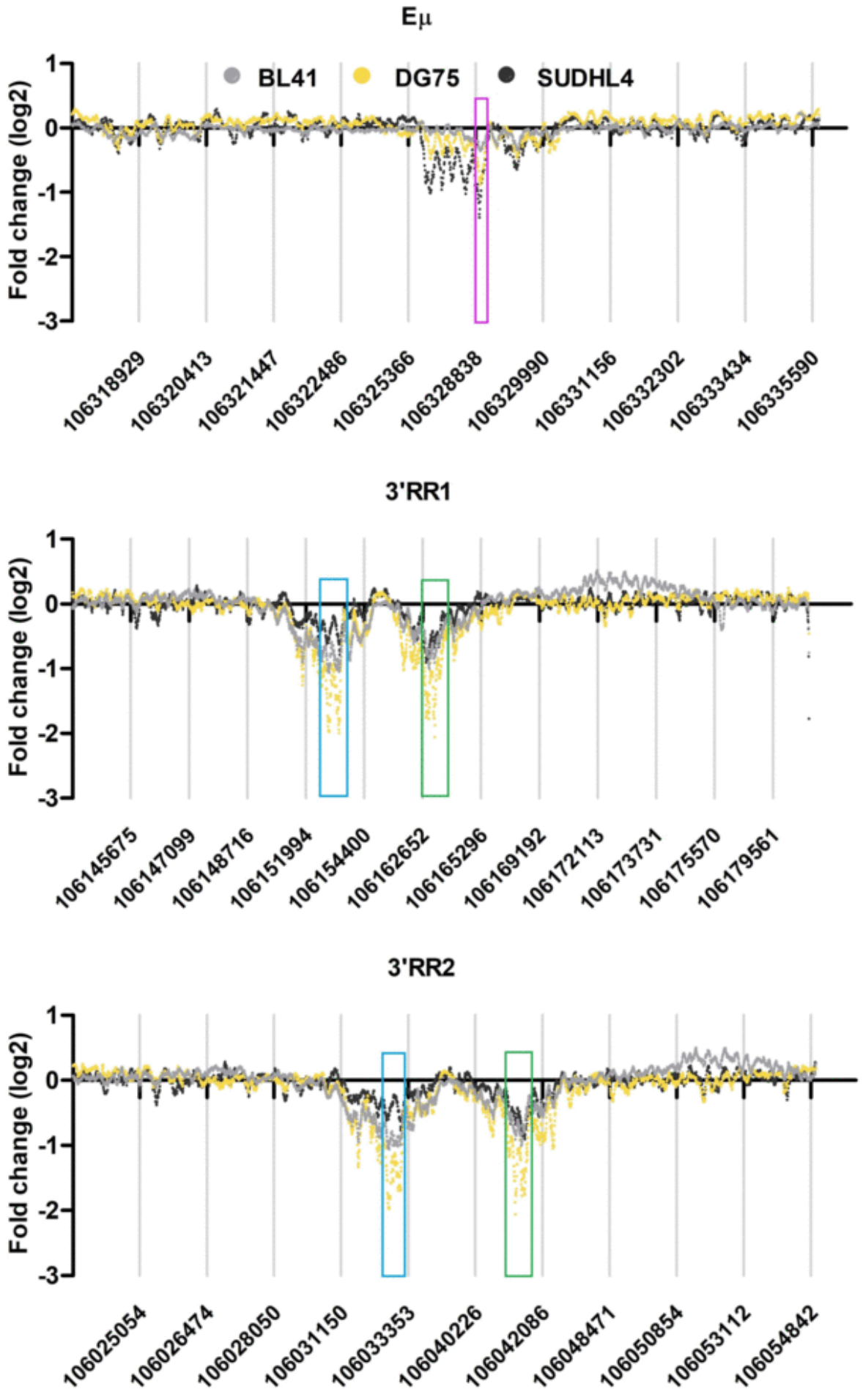
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DG75
SUDHL4
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-2
-3
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106322486
106325366
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Fold change (log2)
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3'RR2
Fold change (log2)
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Figure 1
CRISPR interference screen of the IGH enhnacers in NHL cell lines. Fold change values of 20 consecutive sgRNA calculated using the sliding window approach. Colored boxes mark regions identified as essential for cell survival.

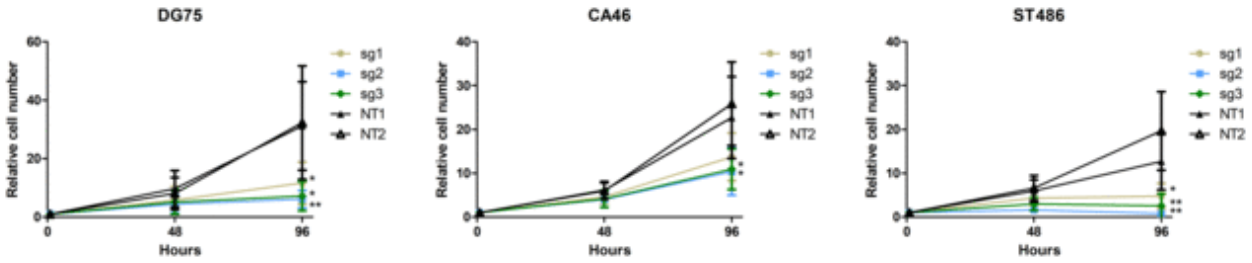

Figure 2
Cell viability upon targeting of the IGH eRNA with Cas13d. Shown are mean values and SD from three independent experiments, each performed in triplicate. *, P < 0.05; **, P < 0.01; Student's t-test.

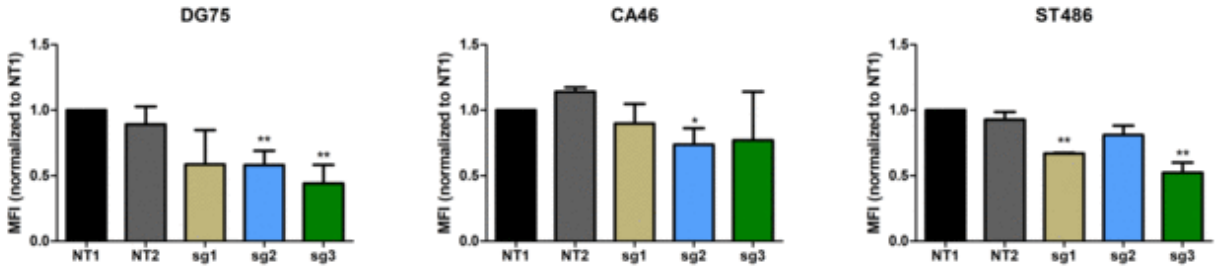

Figure 3
Mean fluorescent intensity of surface IgM. Shown are mean values and SD from three (DG75 and CA46) or two (ST486) independent experiments. *, P < 0.05; **, P < 0.01; Student's t-test.