CD21/CR2 as a possible novel target gene of Myc in hematopoietic cell lines

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c-Myc is a transcription factor of the family of basic-helix/loop/helix-leucine zipper proteins. It is found deregulated in more than 50% of human tumors, including Burkitt lymphoma, a human B-cell tumor, where Myc is chromosomally translocated. There is an African epidemic version of Burkitt lymphoma (BL) associated with the infection of Epstein-Barr virus (EBV) a transforming human herpesvirus with oncogenic potencial. In fact, there is another subtype of BL associated with human immunodeficiency virus (HIV) but it is unclear whether these subtypes use similar or divergent oncogenic mechanisms.

CD21/CR2 is the correceptor of B-cell receptor (BCR) and serves as receptor for EBV. We explored whether CR2 could be a potential Myc target gene. We have used several hematopoietic cell lines (Raji, derived from Burkitt lymphoma, and K562, derived from chronic myeloid leukemia) in which we have measured CR2 mRNA levels by qPCR upon different conditions of Myc expression. Our results indicate that Myc overexpression in K562 cells results in CR2 overexpression and this effect is reproduced in the absence of protein synthesis. Moreover, in Burkitt cells, the drug JQ1 (which impairs Myc transcription) resulted in CR2 mRNA decrease. The bioinformatic analysis of the ChIP-seq data of "ENCODE" project revealed that Myc is bound to the CR2 promoter. Altogether the data strongly suggest that CR2 is a novel Myc target gene and this may help to explain the association of BL in EBV infection.

Material and Methods

Cell lines

Raji: cell line derived from a Nigerian 40 age patient with Burkitt lymphoma. Presents the Myc 8q24 translocation and is Epstein Barr positive.

KMycJ: cells derived from K562 cell line with a $ZnSO_4$-inducible Myc allele. K562 is a derived cell line from a CML patient.

KMycER: is a K562 derived cell line containing the chimerical MycER protein which can be activated with 4HT. Myc-ER is a fusion protein in which the ligand-binding domain of the estrogen receptor is fused with the carboxyl terminus of Myc.

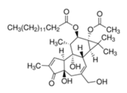
Techniques:

Transcriptional expression levels were measured by Real Time-quantitative PCR (RT-qPCR) and protein expression were measured by Western-blot (WB) assay.

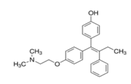
Treatments

JQ1: small molecule that inhibits the individual BET family bromodomains. The cells were treated with three different concentrations of JQ1: 0,25, 0,5 and 1µM. Control cells were treated with DMSO.

Cycloheximide: antibiotic that inhibits protein synthesis. The cells were treated with 50µg/ml of Cycloheximide for 6 hours.

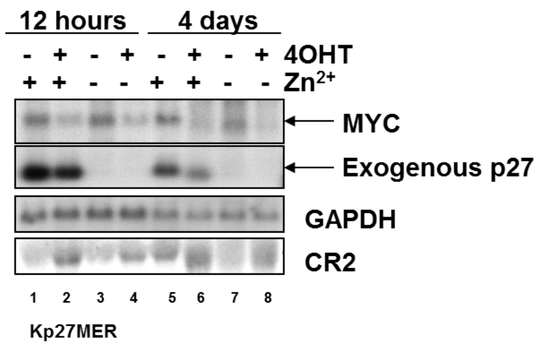


TPA: (12-O-Tetradecanoylphorbol-13-Acetate) small molecule used for hematological cancer treatment which provoke cell cycle arrest at low concentrations. The cells were treated 12 hours with TPA at 10nM.



4-Hidroxitamoxifen (4OHT): the active metabolite of tamoxifen which binds estrogen receptors (ER). We treated cells with 200nM of 4OHT for the activation of the MycER protein.

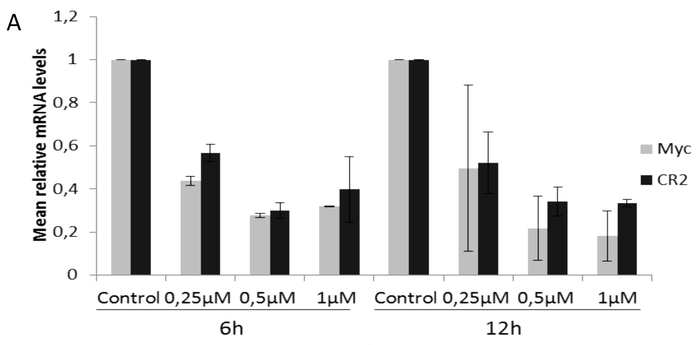
1. Previous results.

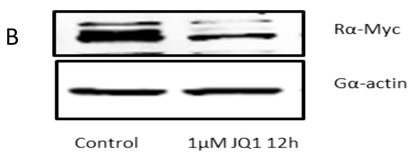


Kp27MER derived from K562 cell line with chimeric protein Myc-ER inducible by Tamoxifen and p27 condicional induction by $ZnSO_4$. Control RNAs of microarray experiment and Myc effect over CR2 gene expression. Northern blot of Kp27MER cells treated with $ZnSO_4$ and 4OHT during 12 hours and 4 days with probes of Myc and CR2. GADPH were used as loading control. (J. C. Acosta, PhD Dissertation).

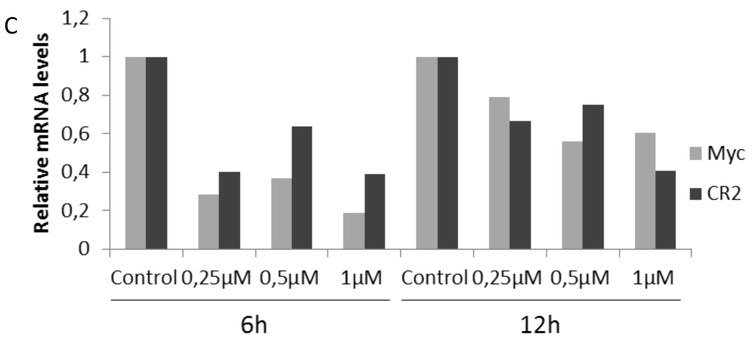
Myc induces CR2 at the transcriptional level, suggesting a new potential Myc target gene

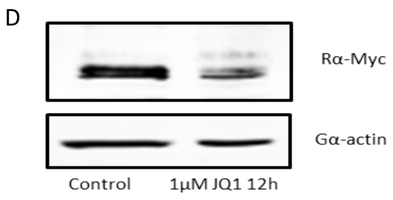
2. Myc downregulation after treatment with JQ1 represses CR2 expression in Raji and Raji derived cell lines





Raji cells treated with 0,25 µM of JQ1 showed decreased Myc expression after 6 hours. This decrease is more significant as we increase the concentration JQ1, although the difference between 0,5 and 1 µM is less obvious than between 0,25 and 0,5 µM. After 12 hours of treatment the cells showed the same gradually decrease of Myc expression and so did CR2. The highest concentration of JQ1 is consistent with lower levels of Myc as confirmed by qPCR A) and WB B).



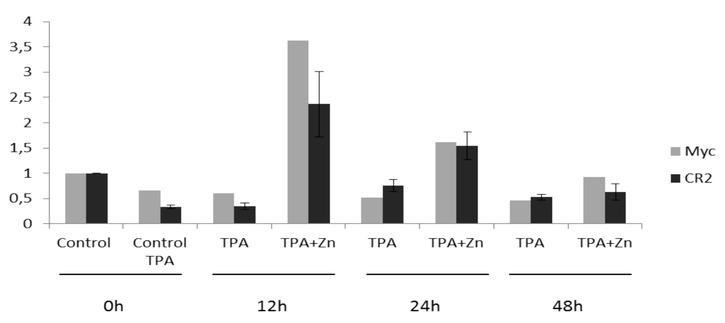


Since JQ1 affects the activity of Myc promoter, Raji6B cells were treated in order to compare JQ1 activity over a different promoter as a control. However it also showed similar inhibition of the exogenous Myc transcription as Raji parental cells at 6 hours, although after 12 hours of treatment Myc expression tends to recover, independently of the concentration. CR2 expression correlates with Myc downregulation at 6 hours and appears to stabilize at 12 hours as shown in C). D) WB showing Myc downregulation at the protein level after JQ1 treatment.

The highest concentration of JQ1 is consistent with low Myc expression at RNA and protein levels, as confirmed by PCR and Western-blot. It also correlates with lower levels of CR2, confirming that Myc inhibition by JQ1 affects CR2 expression.

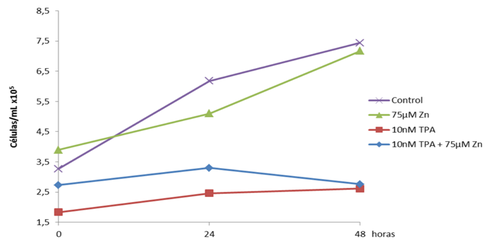
3. Myc upregulation increase CR2 expression

A



A) KMycJ cells treated with TPA showed decreased Myc levels, maintained along time. Cells treated with $ZnSO_4$ in presence of TPA showed an increase of CR2 expression which decreases gradually at 24 and 48 hours after treatment, consistent with Myc induction.

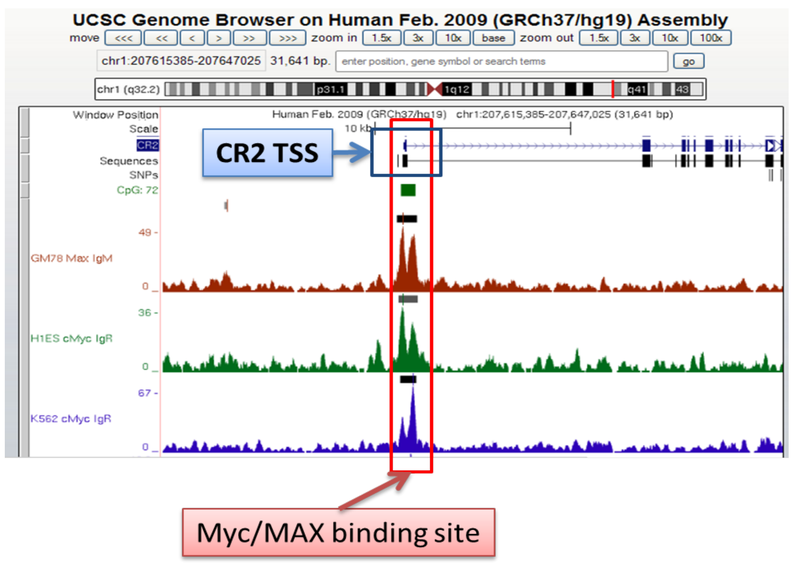
B



B) Control cells and cells treated with Zn^{2+} presented the same growing pattern, however TPA treatment produced growth arrest in control cells. Myc induction by Zn2+ is not able to restore the antiproliferative arrest caused by TPA, therefore this is an ideal system to study Myc target genes independently of cell proliferation.

Myc induction by Zn^{2+} upregulated CR2 expression independently of the proliferating state of the cell.

4. Myc binds CR2 promoter

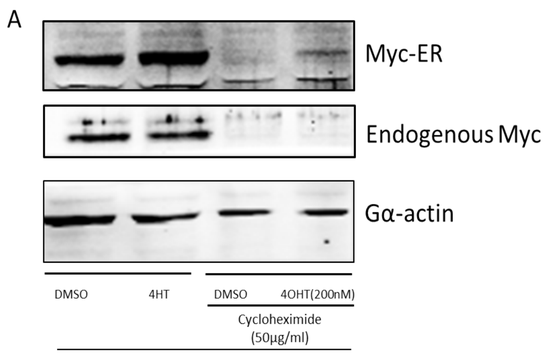


According to the Chip-Seq data from the "ENCODE" project Myc binds the CR2 promoter al the Transcription Start Site (TSS) as shown in the figure. These data also shown that Myc partner (Max) is bound in the same region.

Besides CR2 promoter contains two canonical E-boxes, one at the TSS (17bp upstream the TSS) and the other one in the first intron.

5. CR2 expression is directly regulated by Myc

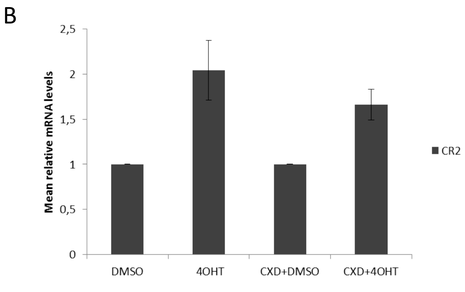
A



A) KMycER cells were harvested after 6 hours of treatment with 4OHT and Cycloheximide. Control cells showed stabilization of Myc-ER protein after 6 hours of treatment with 4OHT by WB. The cells treated with cycloheximide showed the inhibition of protein synthesis as expected, although Myc-ER activation by 4OHT makes it more stable even in presence of Cycloheximide.

B) qPCR analysis showed that activation of Myc-ER by 4OHT correlates with an increase of CR2 expression at transcriptional level. In presence of Cycloheximide we could still see an increase in CR2 levels after Myc-ER activation.

B



CR2 regulation due to a intermediate protein is avoid by inhibition of protein synthesis, confirming the hypothesis that CR2 is a direct target of Myc.

Conclusions:

- **Myc transcriptional levels are correlated with CR2 expression in presence of JQ1.**
- **This correlation is independent of cell proliferation.**
- **Myc binds to CR2 promoter in its canonical binding sites and regulates directly its transcription.**

Future approaches: **we tried to silence Myc expression by lentiviral infection with shMyc and with siRNAs but without success so far. Therefore we will try to silence Myc in Raji cell line and other Burkitt lymphoma cells to confirm the results obtain until now.**

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