BCL6 and LRF Crosstalk in Follicular Lymphoma

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Motivation
B cell Lymphoma 6 (BCL6) and Leukaemia/Lymphoma related factor (LRF) are Pox-proteins over-expressed in some types of Non-Hodgkin's lymphoma. BCL6 is located on chromosome 3 in the breakpoint affecting 3q27 band which is the most frequent translocation in Non-Hodgkin's lymphomas. This gene is a transcriptional repressor whose principal effect is to thwart the response to DNA damage by directly inhibiting both p53 and the cell cycle inhibitor p21. In lymphoma cells BCL6 prevents apoptosis induced by DNA damage. LRF, encoded by the Zbtb7a gene, also known as Pokemon, is a transcriptional repressor involved in many cellular processes as viral infection, differentiation, inflammation and oncogenesis. LRF plays an important role as proto-oncogene in Non- Hodgkin's lymphomas: LRF indirectly inhibits p53, by repressing p14/ARF with consequent activation of MDM2 followed by p53 degradation. LRF is often aberrantly over-expressed in association with BCL6 in diffuse large B cell lymphoma (DLBCL) and follicular lymphoma (FL), the commonest types of Non- Hodgkin's lymphomas. It has been demonstrated that microRNAs, a class of endogenous 22-25 nt single stranded RNA molecules, regulate target gene expression at the post-transcriptional level by binding with imperfect complementarity to specific regions of the 3'UTR of the target mRNA and act by repressing its translation. Recently miRNAs were shown to participate in the network of oncogenes and tumour suppressors, behaving either as tumour suppressors or as oncogenes, with the consequent implication of their potential use as novel anti tumourigenic drug. For these reason we investigated the role of miRNAs in the complex network connecting BCL6 and LRF in follicular lymphoma.

Results
In this work we focused our attention on the possible correlation between BCL6 and LRF in DOHH2 cells taking into consideration miRNAs which potentially link these two oncogenes. Using our data and data present in the literature, we reconstructed a hypothetical circuit connecting BCL6 and LRF. A negative feedback loop appears to link BCL6 to LRF, however in DLBCL and FL both genes are over expressed; these data strongly indicate that the negative feedback loop is bypassed in tumour cells to favour high proliferation rate. Different approaches were used to modulate the expression of BCL6 and LRF in Dohh2, a cell line derived from Follicular Lymphoma. A) Dohh2 cells were treated with etoposide, a chemotherapeutic agent that is able to reduce both BCL6 and LRF. Our results show that the reduction of BCL-6/LRF cells is followed by cell proliferation
block and over-expression of miR-145, a tumour suppressor miRNA, under p53 control, which targets c-myc. Interestingly while c-myc was down regulated as expected, E2F1 resulted to be up regulated. B) To verify whether miR-145 could substitute for etoposide treatment miR-145 was transiently over expressed in Dohh2 cells. Although the network was influenced by miR-145 and both BCL6 and LRF were down regulated, no change in the proliferation rate of Dohh2 cells was found. Unexpectedly p53 was also markedly down regulated which could in part explain why cell proliferation was not inhibited notwithstanding BCL6/LRF decrease. C) In order to see the effect of a prolonged increase of miR-145, stably BCL6 silenced Dohh2 cells were used. In this case miR-145 was stably up regulated due to p53 increase and LRF was down regulated, yet again no effect on cell proliferation was observed. Our results clearly show that in the tumour Dohh2 cells BCL6 and LRF are always co-regulated (contrary to the hypothetical network); however their concomitant down regulation is not sufficient to determine cell proliferation block as cells appear to find a new equilibrium to counteract these anti-proliferative signals. We suggest that the down regulation of BCL6/LRF must be concomitant to the up-regulation of the tumour suppressor p53 and the proto-oncogene E2F1 to influence the cell cycle and that miRNAs (such as miR-145 and miR-20) are part of these regulatory mechanisms.

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