Background: The gene fusion between ETV6 and RUNX1 (E/R) is a frequent translocation in acute lymphoblastic leukemia (ALL). Patients carrying this translocation are associated with a good prognosis and excellent molecular response to treatment. However, up to 20% of cases relapse. Furthermore, the treatment of some relapse cases is associated by resistance to treatments such as glucocorticoids, and these patients must be treated with stem cell transplantation. Recent studies suggest that ETV6/RUNX1 (E/R) plays a role in the initiation of leukemia and is also essential for disease progression and maintenance, through the deregulation of different molecular pathways that contribute to leukemogenesis, such as the upregulation of PI3K/AKT/mTOR pathway.

Methods: 1) Based on CRISPR/Cas9 system, sgRNAs directed towards the fusion gene were designed to produce indels modifying the oncogene ORF and, therefore, the expression of the protein. Tumor cells (REH cell line expressing E/R fusion gene) were electroporated (Amaxa nucleofector, Basel, Switzerland) and sorted by flow cytometry (pCR and Western Blot were used to check ER mRNA expression and downstream targets expression). 2) Cell viability was measured by MTT cell proliferation assays in E/RKO cells and E/R positive cells treated with copanlisib, a PI3K inhibitor, (10 nM) and prednisolone (250 µM). 4) In a xenograft model, E/R positive cells (right flank) and E/RKO cells (left flank) were subcutaneously injected in 16 NOD/SCID/IL2 receptor gamma chain null mice. Excised tumors were sampled just after sacrifice (left flank) were subcutaneously injected in 16 NOD/SCID/IL2 receptor gamma chain null mice. Excised tumors were sampled just after sacrifice.

Results: 1) A total loss of E/R expression was observed in different E/R KO clones established by single cell, demonstrating an effective disruption of the oncogene in REH cells. Moreover, a decrease of downstream target expression levels such as phospho-Akt (66%), BCL-2 (48%) and BCL-XL (52%) was observed. 2) Abrogation of E/R fusion gene showed a significantly decrease of oncogenic potential. 3) Tumor cells showed a higher sensitivity to copanlisib alone and in combination with prednisolone, in vitro and in vivo. Mice injected with E/RKO cells did not generate tumors or generated significantly smaller tumors than those generated by E/R positive cells. Furthermore, a higher rate of mitotic activity was observed in tumors from E/R positive cells (62 vs 20; p = 0.006). 3) Tumor cells showed a higher sensitivity to copanlisib and combination of copanlisib and prednisolone in vitro after E/R depletion. Treatment with copanlisib raised to decrease the cell viability up to 35% in E/R KO cells vs. 55% in E/R positive cells (p < 0.05). In the same way, combination of copanlisib and prednisolone was more effective in E/R KO cells (26% vs 34% of cell viability, p < 0.05).

Summary/Conclusion: Avoiding the E/R fusion gene expression reduces significantly the oncogenic potential of ALL cells (REH, E/R positive) both in vitro and in vivo. E/RKO cells also showed an increased sensitivity to copanlisib alone and in combination with prednisolone, suggesting E/R expression could be involved in the prednisolone resistance observed in some patients. These results showed that E/R plays an important role in the maintenance of the leukemic phenotype. The fusion gene could therefore become a potential therapeutic target.

Acute lymphoblastic leukemia - Clinical

Differential Expression Patterns of Specific Long Noncoding RNAs and Competing Endogenous RNA Network in Acute Lymphoblastic Leukemia

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Background: Increasing evidence has demonstrated that long non-coding RNAs (lncRNAs) play an important role in the competitive endogenous RNA (ceRNA) networks in that they regulate protein-coding gene expression by sponging microRNAs (miRNAs). However, the roles of specific lncRNA and its related competing endogenous RNAs (ceRNA) network in acute lymphoblastic leukemia (ALL) are not fully understood.

Aims: The aims of this study were to use RNA expression profile bioinformatics data from cases of ALL from the Cancer Genome Atlas (TCGA), the Kyoto Encyclopedia of Genes and Genomes (KEGG), and the Gene Ontology (GO) databases to construct a ceRNA network of miRNAs, lncRNAs, and mRNAs.