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Zfp521 promotes B-cell viability and cyclin D1 gene expression in a B cell culture system

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Leukemia originates due to errors in the hematopoietic differentiation of stem cells into mature lymphocytes. Developmental control of early B lineage cell differentiation is exerted by a regulatory network of key transcription factors. The high frequency of B-lineage lymphoma in mice with a proviral insertion at the Evi3 locus suggests that it alters the gene expression near the insertion site to promote B-lineage lymphoma. Due to its zinc finger motifs, the gene at the Evi3 insertion site has been renamed Zfp521 in mice and ZNF521 in humans. The role of Zfp521 in B-cell differentiation, and the mechanisms by which it leads to leukemic transformation, are unclear. Mouse lymphoblast cells were cultured in RPMI 1640 medium supplemented with 2 mM L-glutamine, 0.05 mM 2-mercaptoethanol, 15% FBS, 5% penicillin, 5% streptomycin at 37°C with 5% CO2. Four independent Zfp521 shRNA expression vectors with a CMV-Green Fluorescent Protein marker were combined at equal concentration for transfection. A vector containing scrambled Zfp521 shRNA sequence and an empty vector lacking any shRNA sequence were used as controls. 1 µg plasmid DNA was transfected into 1×105 BCL1 cells with FuGENE HD in OptiMEM Media. Viability assay was conducted. Absorbance was recorded at 490 nm. BCL1 cells were trypsinized and cells were re-suspended in BCL1 media. An equal amount of cell suspension and trypan-blue solution was mixed together. Cells were visualized under light microscopy. The total cell number and number of dead cells (stained blue) were determined. Caspase activity was assessed using Apo-ONE Homogenous Caspase-3/7 Assay (Promega; no: G7792). Absorbance was recorded at 490 nm. Real-time quantitative PCR analysis was performed and expression was analyzed using the ΔΔCT method. Results were analyzed by t test for statistical significance. Site-directed mutagenesis primers were designed using software from New England BioLabs: Forward primer: 5'-TGCACAGCTGAGACAGCTGCC-3' and Reverse primer: 5'-CAGCGTCCTCCTCCAACTC-3', followed by rescue assay.

Biography

Salma Al Dallal is working as a Faculty Member in Life Sciences Department, University of Manchester, Manchester, UK. Her research papers are published in various journals and she also participates in Medical programs.

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