

Retrotransposons in circulating free DNA from cancer patients

Retrotransposons in zirkulierender freier DNA von Krebspatienten

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Introduction: L1 (Long interspersed nuclear element 1) retrotransposons are part of non-long terminal repeat (non-LTR) retrotransposons that are positioned throughout the genome and comprise 17% of it. Through transposition, L1 is involved in genome rearrangement, thus regulate gene expression of specific genes or induce mutations. According to literature and experimental data, ORF2, which contains both endonuclease and reverse transcriptase activities, is expressed higher in cancer cells and in circulating tumor cells (CTCs). Apart from CTCs, the circulating free DNA (cfDNA), includes information about the tumor, and therefore might be useful tool in cancer treatment and progression of the disease. The present study aimed to identify the existence of L1 in cfDNA from cancer and healthy individuals.



Figure 1: Electrophoresis of PCR products

Materials & Methods: Blood was collected from 20 cancer patients in specific tubes and protocol for cfDNA isolation was performed from plasma samples. Patients represented different types of cancer (breast, colon, lung, prostate), while two healthy individuals (non-cancer) were also used in the study. In all cases, genomic DNA was extracted from blood, to be used as control in cfDNA isolation, as well as for testing the existence of L1 in gDNA. Real-Time PCR experiments were performed for L1-ORF1, L1-ORF2, and the housekeeping gene ACTB. The experiments were performed in triplicates, and the products were analyzed by agarose electrophoresis.

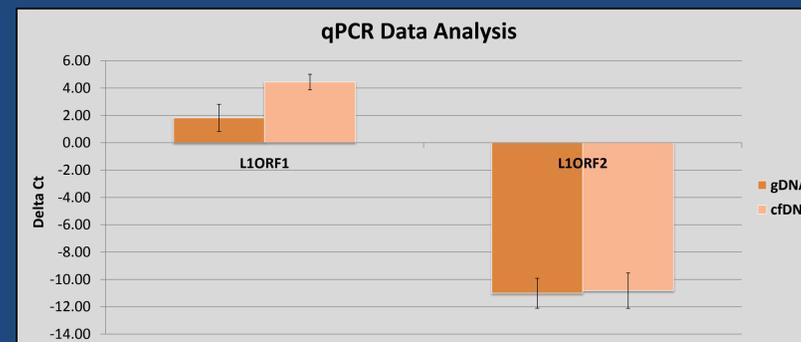


Figure 2: qPCR analysis of cfDNA and gDNA. A lower DeltaCt indicates higher amount of DNA.

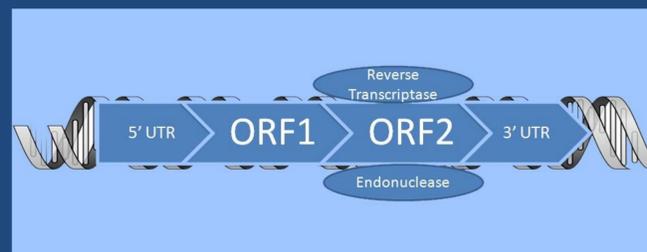


Figure 3: L1 DNA Structure

Results: The analysis of L1 exhibited that L1-ORF1 and L1-ORF2 exist not only in genomic DNA but also in cfDNA. The amount of PCR products revealed however that L1ORF2 was in higher quantities in gDNA than in cfDNA. In addition, L1ORF2 quantity was higher in cancer samples when compared with normal individuals.

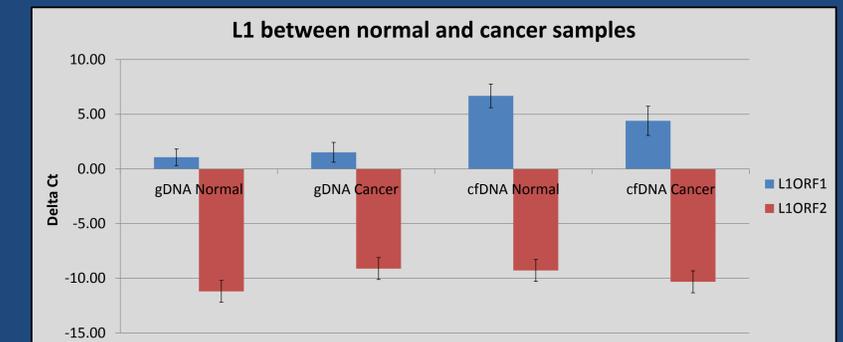


Figure 4: qPCR analysis of cancer and normal samples. A lower DeltaCt indicates higher amount of DNA.

Conclusion: Taking everything into consideration it has been approved that L1 exist not only in genomic DNA but also in cfDNA. L1 has been implicated in different cellular processes, through gene expression regulation; therefore it might be used both in gDNA as well as in cfDNA analysis. The study in more samples is essential to be used at clinical level.

Disclosure of Potential Conflicts of Interest

None of the authors of the above study has declared any conflict of interest

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