**OTR.27 - Screening for rearrangements in RB1 / 13q14 through real-time PCR**

Renata Mendes de Freitas*.

1Fiocruz - Fundação Oswaldo Cruz.

**Introduction:**

Patients with deletion of chromosomal region 13q that includes the RB1 gene show retinoblastoma (RB) and variable clinical features. About 5-15% of the patients with RB are heterozygous for a gross deletion that includes the whole or substantial parts of RB1 gene.

We have designed a method based in real-time PCR for search of deletions / duplications of RB1 gene. The specificity, sensitivity and clinical utility of the assay were demonstrated in detecting allele-specific copy number variation, and can be useful for analysis relative copy number.

**Objective:**

We have selected, in addition to the RB1 gene, two other genes (SUCLA2 and MED4) that are adjacentl located to RB1 in chromosomal region 13q14.2.

**Methodology:**

Genomic DNA was isolated from peripheral blood samples. The amplified segments were analyzed by relative quantification, relative copy number method (2-\(^{10}\)). Each plate contained an internal control (ALB gene) and a trisomic sample. The CT's (cycle threshold) values obtained were used to calculate the relative copy number of each sample. All reactions were performed with Sybr Green (Invitrogen*). The \(2^{-\Delta\Delta CT}\) of each sample were estimated.

Five samples of retinoblastoma patients with partial or total RB1 deletion detected by MLPA were used for validation. All samples were validated by real-time quantitative PCR.

**Results:**

Nine retinoblastoma patients carried complete deletion of RB1 gene were identified by the MLPA technique, among 66 retinoblastoma patients tested. These deletions comprise about 10.61% of the mutations identified in retinoblastoma.

**Conclusion:**

Thus, the relative quantification real-time PCR technique to investigate deletions in the RB1 gene becomes advantageous.

**Keywords:** retinoblastoma; Real time PCR; 13q14