[788] **DOWNREGULATION OF NF-KB1 ENHANCES THE RADIOSENSITIVITY OF RENAL CELL CARCINOMA**

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Introduction: Clear cell renal cell carcinoma (ccRCC) accounts for ~80% of all renal cell carcinomas (RCC) and has the Von Hippel–Lindau (VHL) tumor suppressor gene mutated. The lack of VHL protein leads to a constitutionally active Hypoxia Inducible Factor (HIF) pathway that confers both chemoresistance and radioresistance for renal tumor. HIF pathway is known to interact with the transcription factor nuclear factor kappa B (NF-kB). Increased NF-κB activity is associated with the development and progression of RCC (IKEGAMI A, TEIXEIRA LF. BRAGA MS et al. The American Society for Cell Biology 2016; 26: 3948-3955).

Objective: Evaluate the synergistic effect of NF-kB1 knockdown and ionizing radiation in murine renal adenocarcinoma cell line.

Methods: The murine renal adenocarcinoma cell line (Renca cells) (ATCC, USA) was cultured in RPMI 1640 supplemented with 10% FBS and penicillin/streptomycin. Lentiviral shRNA vector was used to knockdown of NF-KB1 gene in Renca cells, as described previously (1). In the clonogenic cell survival assay, the cells were irradiated by 60Co source in the range from 0 to 10 Gy, using the GammaCell 220 – Irradiation Unit of Canadian-Atomic Energy Commision Ltd. (CTR-IPEN). After 10-14 days of culture, cell colonies were fixed and stained with formaldehyde 4% and rhodamine B 2% and counted. To assess cell viability, tetrazolium [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-MTS] was performed within 24 hours after irradiation at a dose of 10Gy. The survival variables α e β were fitted according to the linear quadratic equation ($SF=\exp[-\alpha D-\beta D2]$); SF=survival fraction, D=dose of irradiation and P value was determined by F test. Multiple comparisons were assessed by One-way ANOVA followed by Bonferroni´s tests with GraphPad Prism version 6.0 software. $P< 0.05$ was considered statistically significant. Data are shown as the mean ± SD.

Results: The Renca-shRNA-NF-kB1 cells were found to be significantly more radiosensitive than controls - Renca-WT and Renca-Mock, ($P<0.001$ vs Renca-Mock). The ratio α/β was increased in Renca-shRNA-NF-kB1: -0.177±0.677 compared with 7.368±1.833 and 11.960±5.240 of the Renca-WT and Renca-Mock, respectively. There was no significant difference in the survival fraction between Renca-WT and Renca-Mock groups. The lethal dose 50% (LD50) of Renca-WT was 3.33 Gy and Renca-Mock was 3.288 Gy whereas for the Renca-shRNA-NF-kB1 group it was 2.08 Gy. Corroborating these data, the Renca-shRNA-NF-kB1 showed reduction of 16.75±0.06% in the viability when compared to the Renca-Mock ($P<0.001$).

Conclusion: The knockdown of NF-kB1 gene mediated by shRNA on Renca cells led to a decrease in the radioresistance. Therefore, this gene can be a therapeutic target for CCR treatment.

Financial Support: FAPESP and CNEN.

Key words: Radioresistance; nf-kb1; renal adenocarcinoma