Tissues, Pathology, and Diagnostic Microscopy

LS.2.P075
Evalution of Rapamycin Effects on MDA-MB 231 Breast Cancer Cell Line Using Immunohistochemistry and TUNEL Methods

E. Turkoz Uluer[1], M. Kıvanc[1], Y. Sarıca[1], G. Evirgen[1], S. Inan[1]

[1]Celal Bayar University, Histology and Embryology, Manisa, Turkey

drelginturkoz@gmail.com
Key words: MDA-MB 231 breast cancer cell line, Rapamycin, PI3K, Apoptosis, Immunohistochemistry

Breast cancer is the most common cancer type in the women [1]. PI3K/AKT/mTOR and Ras/Raf/MEK/ERK pathways are frequently dysregulated in cancer [2]. In this study, we aimed to evaluate the effects of Rapamycin, which is an inhibitor of mTORC1, on the MDA-MB 231 breast cancer cell line with primary antibodies involved in PI3K, ERK and apoptotic signal pathways using indirect immunohistochemistry and TUNEL methods.

MDA-MB 231 breast cancer cells were cultured in RPMI-1640 medium containing 10% fetal bovine serum, 1% L-glutamine and 1% Penicillin/Streptomycin. After growing cells on 24 well-plate, the IC_{50} dose of Rapamycin was applied to the cells and the effect of 24^{th} hour was evaluated. For the indirect immunohistochemistry and TUNEL methods, cells were fixed in 4% paraformaldehyde. Cells were incubated with anti-mTORC1, anti-mTORC2, anti-pAKT, anti-PI3K, anti-ERK, anti-IGF, anti-caspase3, anti-caspase8, anti-caspase9, anti-APAF primary antibodies with the 1:100 dilution for immunohistochemistry and TdT for TUNEL method. Diaminobenzidine was applied to the cells as chromogen and for the background staining, Mayer's hematoxylin was used. Cells were covered with mounting medium, then viewed under light microscope (Olympus BX40). The distribution of immunohistochemical intensities of primary antibodies were scored as mild (+), moderate (++), strong (+++) and very strong (++++). After counting the percent of positive staining cells, statistical significance was determined by assessment of differences using the ANOVA test. Significance was defined as $p<0.05$.

According to the immunohistochemical evaluation, mTORC1 and IGF immunoreactivities were observed to be very strong, pAKT, PI3K and ERK immunoreactivities were strong in the MDA-MB 231 breast cancer cell line (control group); while mTORC1, PI3K and IGF immunoreactivites were observed to decrease, on the other hand pAKT and ERK immunoreactivities were observed to increase in Rapamycin treated group (Rapamycin group). mTORC2 immunoreactivity was observed to be very strong in both control and Rapamycin groups (Table 1, Figure 1).

While caspase3, 8, 9 and APAF immunoreactivities were observed as moderate in control group, caspase3 and APAF immunoreactivities were observed as strong and caspase8, 9 immunoreactivities were very strong in Rapamycin group. In concordance with the immunohistochemistry results there were much more TUNEL positive cells in Rapamycin group when compared with the control group ($p<0.05$) (Figure 1, Table 1).

In this study, we showed the activation of PI3K/AKT/mTOR and ERK-related molecular signal pathways in MDA-MB 231 human breast cancer cell line using indirect immunohistochemistry and TUNEL methods. The increased immunoreactivities of pAKT and ERK might be related with negative feedback of mTORC1 inhibition by Rapamycin [3]. The intensity of immunoreactivities of caspase3, 8, 9 and APAF were higher in Rapamycin treated group compare to control group ($p<0.05$). Both intrinsic and extrinsic pathways of caspase activation might be involved in Rapamycin caused apoptosis. In conclusion, these pathways may play an important role in cancer pathogenesis and new drug development for PI3K and mTORC2 inhibition is required.

1 D.R. Youlden, S.M. Cramb, N.A.M. Dunn et al., Cancer Epidemiology 36 (2012), p. 237-248.
2. S. Grant, JCI 118 (2008), p. 3003-3006.
3. L.F. Hernandez-Aya, A.M. Gonzalez-Angulo, The Oncologist 16 (2011), p. 404-414.

ANTIBODY NAME	Control Group	Rapamycin Group	ANTIBODY NAME	Control Group	Rapamycin Group
mTORC1	++++	++	IGF	++++	+++
mTORC2	++++	++++	Caspase3	++	+++
pAKT	+++	++++	Caspase8	++	++++
PI3K	+++	++	Caspase9	++	++++
ERK	+++	++++	APAF	++	+++

Table 1. Intensity of the immunoreactivities of mTORC1, mTORC2, pAKT, PI3K, ERK, IGF, Caspase3, Caspase8, Caspase9 and APAF on Control and Rapamycin Groups

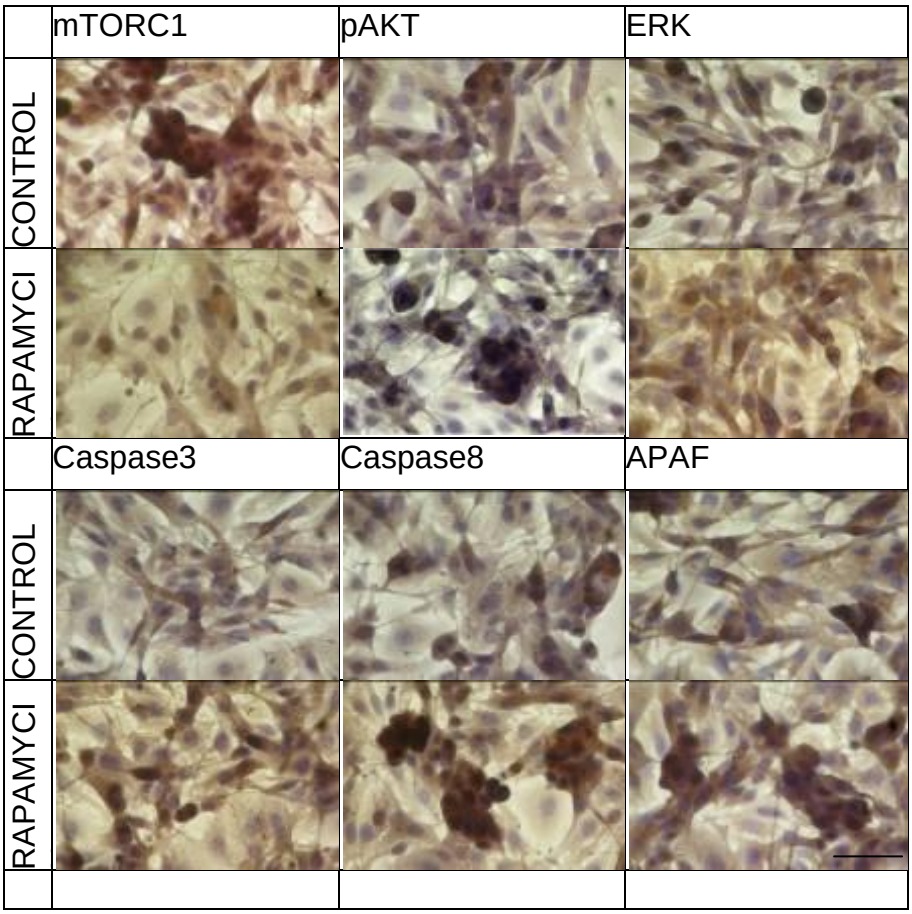

Figure 1. Distribution of mTORC1, AKT, ERK, Caspase3, Caspase8, APAF immunoreactivities of samples from Control and Rapamycin Group. Scale bar: 25µm