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**LS-1-P-6076 The importance of Rapamycine usage on CD 44 and RHAMM expressions on breast cancer cell lines**

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CD44 is a member of superfamily hyaluronan (HA) binding proteins (HABPs) that play a role in cell adhesion, migration, invasion and survival. CD44 and Receptor for HA-mediated motility (RHAMM) are increased during tissue repair and carcinogenesis. Mammalian target of Rapamycin (mTOR) is a serine/threonine protein kinase which belongs to the phosphatidylinositol 3-kinase (PI3K) family. Rapamycine is a macrocyclic antibiotic, has been known to inhibit mTOR by destabilizing the mTOR-Raptor complex. The aim of this study was to examine the effects of Rapamycine on the distributions of CD44 and RHAMM expressions, using indirect immunohistochemistry and RT-PCR methods on non-invasive MCF-7 and invasive MDA-MB 231 breast cancer cell lines.

MCF-7 and MDA-MB231 cells were cultured in RPMI-1640 medium; containing 10% fetal bovine serum, 1% L-glutamine and 1% antibiotic solution in a humidity incubator at 37°C, containing 5% CO2. Cells were grown on cover slips and incubated with Rapamycine for 24 and 48 hours. Cells were immunostained with anti CD44 and anti-RHAMM primary antibodies using avidin-biotin-peroxidase method. Staining intensities were measured by using semi-quantitative method and ANOVA statistical test, to compare the results. The total RNA was extracted using EruX Universal RNA Purification Kit according to manufacturer’s instructions from MCF-7 and MDA-MB-231 cell lines. The cDNA synthesis from total RNA was performed using EurX dART RT-PCR Kit and RT-PCR process from obtained cDNAs was carried out using the Solis BioDyne qPCR Mix Plus.

It was observed that MCF-7 and MDA-MB 231 cells had strong CD44 and RHAMM immunostainings on their cell surfaces and in the cytoplasms, especially in mitotic cells (Figure 1-4). Decreased RHAMM immunoreactivity was detected on MCF-7 and MDA-MB 231 cells in Rapamycine treated groups while CD44 immunoreactivity was detected as decreased on only MDA-MB 231 cells. Compared the gene expression profile of CD44 and RHAMM genes, gene expressions were detected as relatively increased in MCF-7 cells than MDA-MB 231 cells. Decreased RHAMM expression was detected both MCF-7 and MDA-MB 231 cells, while CD44 expression was decreased only in MDA-MB 231 cells in treatment with Rapamycine in RT-PCR method.

CD44 and RHAMM suggested novel prognostic markers for breast cancers and hyaladherins could be used as a target for cancer therapy. Rapamycin is the most well studied mTOR inhibitor and this might be effective on invasive breast cancer treatments in addition to chemotherapy and/or radiotherapy.

References:


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Fig. 1: Immunoreactivity of CD 44 in MCF-7 cells

Fig. 2: Immunoreactivity of RHAMM in MCF-7 cells

Fig. 3: Immunoreactivity of CD 44 in MDA-MB-231 cells.

Fig. 4: Immunoreactivity of RHAMM in MDA-MB-231 cells.