SAĞLIK BİLİMLERİNDE
GÜNCEL AKADEMİK ÇALIŞMALAR-2018

CURRENT ACADEMIC STUDIES
IN HEALTH SCIENCES-2018

VOLUME / CİLT: II

Editör / Editor

Asst. Prof. Dr. Ayhan GÜLER

ISBN 978-9940-540-53-1

EFFET OF PUNICA GRANATUM TO CELL SURVIVAL PATHWAY ON BREAST CANCER CELL LINE

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1. Introduction

Punica Granatum (PG) is a fruit that is native to Iran but it grows in many countries. PG is a medicinal plant that have many pharmacological properties. It has been powerful potential for anti-tumorigenic, anti-inflammatory, antioxidant, anti-proliferative, and anti-angiogenic properties [1]. It has also been shown to be effective in diseases such as cancer, cardiovascular, diabetes, male infertility, Alzheimer and aids. It is known that there are many mechanisms of action including tumor cell proliferation, cell cycle, invasion, and inhibition of angiogenesis [2]. PG is rich in polyphenol, tannin, pedinculgin, ellagic acid, punicic asid, flavonoid, anthocyanin. The amount of polyphenol in PG is known to be higher than many fruits such as grapes, blueberry, and apples [3]. Breast cancer-associated mortality rate is second among women in the world and is found to be more prevalent in less developed regions. PG has been shown to be a promising therapeutic agent against breast tumors. The E-cadherin up regulation of PG and its components showed that tumor cells reduced adherence and decreased tumor cell migration without affecting normal cells. Also recent studies shown that PG inhibits proinflammatory cytokines (IL-8, PDGF-b) and restricts chemotaxis of tumor cells [4]. Erk1 is a serine/threonine kinase that found MAPKinase signal transduction pathway. This cascade responsible for the cell adhesion, cell cycle, cell survival, differentiation, migration and proliferation. Especially the activity of ERK1 pathway is related to many human cancer because of this reason inhibition of this cascade is important for anti cancer activity [5]. PI3K is a lipid kinase that is controlled by many growth factor and responsible for many important cellular events. It has oncogenic potential so it is targeted by human cancer research [6]. mTOR is a serine / threonine protein kinase that is located at a central site in intracellular signal cascades. mTOR activity is controlled by positive and negative upstream regulator. Positive regulator such as growth factors and their receptors transmit signal to mTOR through PI3K/AKT [7]. PI3K/AKT/mTOR

pathway is one of the main signaling pathways necessary to replace normal cellular functions. Since the mutations associated with many cancers have been detected in the pathway, PI3K/AKT/mTOR cascade is one of the most studied. The purpose of this study is investigation the effect of pomegranate fruit on one of the significant pathway that involved in breast cancer.

2.Materials and Methods

MDA-MB breast cancer cell line was used. The cells were grown in RPMI 1640 medium supplemented with %10 fetal bovine serum, %1 penicillin/streptomycin, %1 L-glutamine, %0.5 non-essential amino acid. The cells were incubated in the presence of 5% CO_2 at 37°C and 100% relative humidified atmosphere.

MTT Assay

The cells were seeded in 96- well plates with a $5x10^4$ cell in each well and incubated 24h for %100 confluence. Then the cells were incubated with different concentration of PG (10, 20, 30, 40, 50 µg/ml). PG was dissolved in DMSO. After MTT assay the IC_{50} value was found 18,21µg/ml.

Immunocytochemistry

To detect the expression of PI3K, mTOR and ERK1 specific monoclonal antibodies were used. The cells were fixed with %4 paraformaldehyde and the threated with the 0.1% Triton-X for permeabilisation. Then the cells were blocked with blocking solution and incubated with primary antibodies at 4°C overnight. Then the secondary antibody incubation were done and the cells stained with DAP and counterstained with Mayer's hematoxylin.

3. Results and Discussion

The findings show that PG down regulated the expression of PI3K, mTOR and ERK1 (table 1). The results were statistically significant (table 2). The PI3K/AKT pathway regulates cell survival via phosphorylation of mTOR. In this study PG effect the cancer cell survival via down regulating the important cell survival protein. Some studies that is related with PG showed that ERK1 expression also down regulated in the skin cancer. Also PI3K, mTOR expression is down regulated in lung and colorectal cancer. According to our results PG is very effective for cancer cell death. Because

PG treated breast cancer cells showed down regulation of cell survival protein.

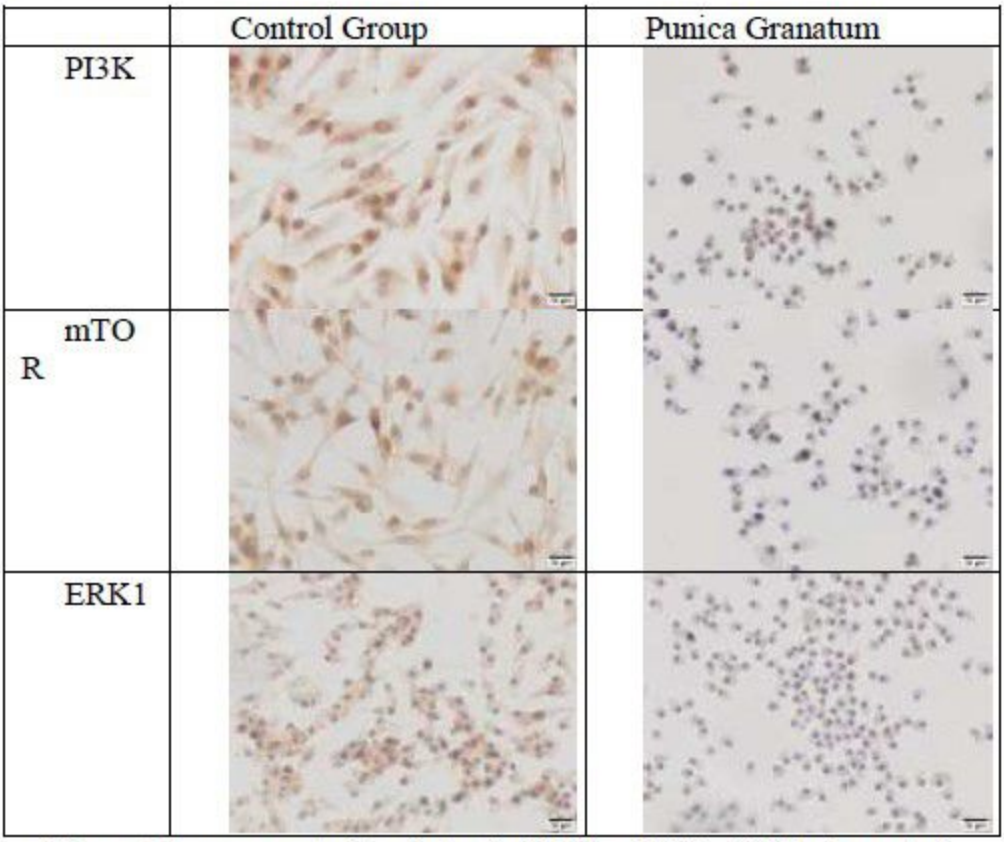

Figure 1:Immunocytochemistry for PI3K, mTOR, ERK1 in control and punica group.

H Score	Control Group	Punica Group	p*
PI3K	285,63±3,85	172,64±7,21	P<0,001
mTOR	224,4±4,47	130,7±5,8	P<0,001
ERK1	240,9±7,85	160,84±8,55	P<0,001

Table 1: h score analysis of PI3K, mTOR, ERK1 in control and punica group.

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