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From Scarcity to Survival: Adaptive Strategies of A549 Lung Cancer Cells Under Serum Starvation

Gunel Mukhtarova[1], Cagla Ozdenizer[1], Deniz Ece[1], Ufuk Mert[1-3], Ayse Caner[1,3,4]

[1]Department of Basic Oncology, Ege University Institute of Health Sciences, İzmir, Türkiye

[2]Atatürk Vocational School of Health Services, Ege University, Izmir, Türkiye

[3]Translational Pulmonary Research Center, Ege University (EgeSAM), Izmir, Türkiye

[4]Department of Parasitology, Ege University Faculty of Medicine, Izmir, Türkiye

INTRODUCTION: Serum plays a critical role in regulating fundamental biological processes, and variations in serum levels can directly influence tumor cell metabolic adaptations, stress responses, and traits associated with tumor progression. The lung adenocarcinoma cell line A549 serves as a widely used *in vitro* model for studying these mechanisms. This study aims to investigate how short- and long-term exposure to different serum concentrations affects A549 cell morphology, proliferation, migration, cell cycle dynamics, gene expression profiles, three-dimensional spheroid formation capacity, and molecular features related to stemness, metastatic potential, and drug resistance. Serum availability is a key determinant of tumor cell behavior, influencing proliferation, differentiation, and survival, while nutrient deprivation triggers adaptive mechanisms that enable cancer cells to endure stress conditions.[1,2]

MATERIAL AND METHODS: A549 cells were cultured under different serum concentrations [1%, 10%, and 20% fetal bovine serum (FBS)], with cells maintained in 10% FBS serving as the control group. To evaluate the effects of serum availability on cellular behavior, various assays were performed, including Giemsa staining for morphology, wound healing assay for migration, flow cytometric analysis of CD44 and CD133 for stemness, XTT assay with flavopiridol for drug resistance, colony formation assay for clonogenic capacity, three-dimensional spheroid formation assay, and reverse transcription quantitative polymerase chain reaction (RT-qPCR) for gene expression profiling.

RESULTS: Significant differences in proliferation and morphology were observed in A549 cells depending on serum concentration. Cell cycle analyses showed that exposure to different serum concentrations caused significant changes in phase distribution. RT-qPCR results revealed significant expression differences in specific genes associated with cellular stress response and proliferation. These findings contribute to elucidating adaptive response mechanisms of tumor cells to nutritional conditions and provide an important approach for the development of *in vitro* tumor biology models.

CONCLUSION: Serum deprivation triggers complex adaptive responses in A549 cells, influencing morphology, migration, stemness, drug sensitivity, and gene expression. Low-serum conditions promoted stress-adaptive and survival phenotypes relevant for modeling tumor microenvironments. These findings suggest that serum availability is a crucial determinant of cancer cell plasticity and behavior. Moreover, long-term adaptation to serum limitation

Corresponding author: Cagla Ozdenizer, e-mail: ozdenizercagla@gmail.com

may mimic the nutrient-restricted conditions of solid tumors, providing a valuable *in vitro* model for studying metabolic flexibility and therapy resistance mechanisms in lung cancer cells.

KEYWORDS: Lung cancer, serum starvation, gene expression, cell migration

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