Seminars in Biotechnology BTEC 592 & BTEC 692

" Deficiency of antiapoptotic Bag-1 increased mesenchymal properties of MCF-7 breast cancer cells"

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Microsoft Teams

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Dr. Pelin Özfiliz Kılbaş completed her undergraduate education in Biology Department of Istanbul University between 2004-2009. She completed his master's degree between 2010-2013 and her doctorate education between 2014-2022 at Istanbul Technical University, Department of Molecular Biology, Genetics and Biotechnology. She has been working as a Research Assistant at the Department of Molecular Biology and Genetics at Istanbul Kultur University since 2011. In 2022, She became a member. She carried out studies on molecular cancer biology and breast cancer. There are 13 international publications, 27 papers presented in national and international meetings and a book chapter. She took part in one internationally supported project between 2017-2019 and 3 national projects between 2011-2022. Between January 30 and February 17, 2023, she participated in the Erasmus+ KA131 Program Personnel Training Mobility in the field of Structural and Computational Biology at Democritus University of Thrace, The Department of Molecular Biology and Genetics in Alexandroupoli, Greece.

The multifunctional Bag-1 protein which is known for its anti-apoptotic role has many critical direct or indirect interaction partners in the cell. According to these interactions, the Bag-1 protein plays an important role in the decision mechanism between survival or death of cancer cells. It has been shown that the change in the expression level of Bag-1 protein in the cell is associated with many different cancer types. In addition, it has been demonstrated that Bag-1 may provide clinical benefit as a prognostic marker in the determination of breast cancer. Elevated levels of Bag-1 are generally associated with breast cancer growth, development, and aggressiveness. In particular, interactions with

the Hsc70/Hsp70 chaperone family are effective in the long-term survival of breast cancer cells under stress conditions. Our previous studies showed that silencing the Bag-1 protein increases drug-induced apoptosis in breast cancer. Although there are several studies on the association of changes in the expression level of the Bag-1 protein with cancer, the biological aspect of the complete deletion of the Bag-1 gene in breast cancer cells is not detailed explained. Therefore, this study investigated the molecular function of the deletion of the Bag-1 gene in MCF-7 breast cancer cells. Bag-1 deficient MCF-7 cells were generated by CRISPR/Cas9, one of the current gene-editing techniques, and the Bag-1 knockout cell models were validated and characterized. Bag-1 knockout cells were grown from a single cell colony, and the deletion of Bag-1 was controlled by the lack of Bag-1 expression in selected cell colonies. Bidirectional Sanger sequencing was performed in the selected colony that showed the loss of Bag-1 expression, and the deletions were determined in both chains of Bag-1 gene regions. Studies showed that the viability, proliferation, and colony formation potentials of MCF-7 cells were decreased in Bag-1 knockout conditions compared to wt cells. To examine the relationship between breast cancer development and Bag-1 deficiency, the phosphorylation profiles of receptor tyrosine kinases were determined which have important roles in breast cancer. The expression level of phospho-Akt Ser 473 was observed markedly increased in Bag deleted MCF-7 cells. Therefore, to examine the relationship between Akt phosphorylation and Bag-1 deprivation, MK-2206, a potential Akt inhibitor, was used to examine the upstream and downstream signaling pathways of the Akt signaling pathway in wild-type, Bag-1 deleted and Bag-1 overexpressed MCF-7 breast cancer cells. Considering the Akt-mediated regulation of actin filaments and the epithelial-mesenchymal transition in cancer, our study showed the expression profiles of β-actin and α-actinin were markedly downregulated in Bag-1 knockout cells, whereas Akt inhibition upregulated the expression of these proteins. The epithelial-mesenchymal transition properties of Bag-1 knockout MCF-7 cells were determined by downregulation of epithelial E-cadherin, and upregulation of N-cadherin expression levels. It was concluded that the deletion of Bag-1 increased stress-induced Akt activation in MCF-7 cells, caused more mesenchymal properties through cytoskeletal remodeling. This study is a novel understanding of the elucidation of the molecular mechanism of Bag-1 deletion in breast cancer cells.