Investigating differences in Polycomb group proteins in a newly developed colorectal cancer EMT model

Zeynep Aleyna ŞAHİN[a], Çetin ILGAZ[b] , Aslı SADE MEMİŞOĞLU[c]*

[a] Department of Biology, Graduate School of Natural and Applied Sciences, Dokuz Eylül University, İzmir, Turkey

[b] Department of Biology General Biology, Faculty of Sciences, Dokuz Eylül University, İzmir, Turkey

[c] Department of Science and Mathematics Education, Faculty of Education, Dokuz Eylül University, İzmir, Turkey

* Corresponding author; asli.memisoglu@deu.edu.tr

ABSTRACT

Colorectal cancer (CRC) is one of the most common malignant tumors worldwide. Colorectal cancer is a type of cancer that is generally curable, but metastasis of the cancer adversely affects the course of the disease. An important condition seen in most malignant carcinomas is that, the cells lose their epithelial and gain mesenchymal characteristics, a process known as epithelial-mesenchymal transition (EMT). For this reason, elucidating the molecular mechanisms of epithelial-mesenchymal transformation is an important step towards understanding metastasis, which is the most important cause of cancer deaths. In this study, a spontaneously differentiating colorectal cancer cell line. HT-29, was used to develop an EMT-MET model. Three types of populations; ancestral group (aHT-29), which is 80% confluent, differentiation group (eHT-29) collected 20 days after growing in galactose media, redifferentiation group (mHT-29) passaged after day 20 and collected at day 4, were generated. Cells were collected on the specified days and the changes in epithelial (E-cadherin, CDX2) and mesenchymal (transgellin, vimentin, fibronectin) markers were examined. The results support that the ancestral group and the redifferentiation group have mesenchymal features, while the differentiation group has epithelial features. In order to elucidate the plastic nature of EMT, changes in the amounts of Polycomb group proteins (EZH1, EZH2, CBX2, CBX4, CBX6, CBX7 and CBX8), in aHT-29, eHT-29 and mHT-29 cells were analyzed. According to the results, EZH2 has decreased and CBX7 has increased eHT29 cells compared to aHT-29 and mHT-29. Differences in the epigenetic regulatory elements would provide potential targets for future diagnostic and therapeutic purposes.

Keywords: Colon cancer, epigenetic, epithelial-mesenchymal transition, polycomb protein

INTRODUCTION

Colorectal cancer (CRC) is one of the most common malignant tumors worldwide. It is also the third most commonly diagnosed cancer in men, the second most common cancer in women, and the fourth leading cause of cancer death worldwide (Wang et al., 2017). Although about half of patients with colorectal cancer can be treated using surgery and multimodal therapy, treatment options are limited, especially for patients with metastases (Arlt & Stein, 2009).

An important condition observed in general malignant carcinomas is that the cells lose their epithelial characteristics and acquire certain mesenchymal characteristics through a biological program called epithelial-mesenchymal transition (EMT). EMT is effective during the metastasis of cancer cells to distant tissues, on the contrary, the MET program is thought to be important in completing the last steps of malignant progression. After carcinoma cells spread to distant tissues and enter new tissue, they usually undergo MET and thus settle in these tissues and complete the last step of metastasis (Pattabiraman vd., 2016). Therefore, EMT and MET can be activated alternately during the metastasis process of carcinoma cells. This EMT-MET transformation demonstrates the plasticity of cell phenotypes. This plasticity is mostly due to the epigenetic changes that occur in this process (Tam ve Weinberg 2013).

MATERIALS AND METHODS

1.CELL CULTURE

HT-29 cells were grown in DMEM growth medium (Glu-medium) containing 25mM glucose and 10% FBS at 37°C and when they reached 80% occupancy to induce differentiation, glucose was replaced with growth medium containing 5mM Galactose and 10% FBS. Cells collected at 80% occupancy were considered **ancestral HT-29 (aHT-29) cells.** Cells were considered day 0 of the differentiation process when they reached 100% occupancy and were grown for 20 days in growth medium containing galactose. These cells were considered as **epithelial HT-29 (eHT-29) cells.** For redifferentiation, on day 21, cells were dispersed with trypsin and seeded in cell culture dishes and grown in growth medium containing glucose. Considering that the cells could enter the epithelial differentiation process again due to the cell density in the redifferentiation groups, the cells that differentiated for 20 days were sowded at different ratios (1:20 and 1:50) after they were collected by trypsin. Cells were collected on day 4. These cells were considered as **mesenchymal HT-29 (mHT-29) cells.**

2.WESTERN BLOT

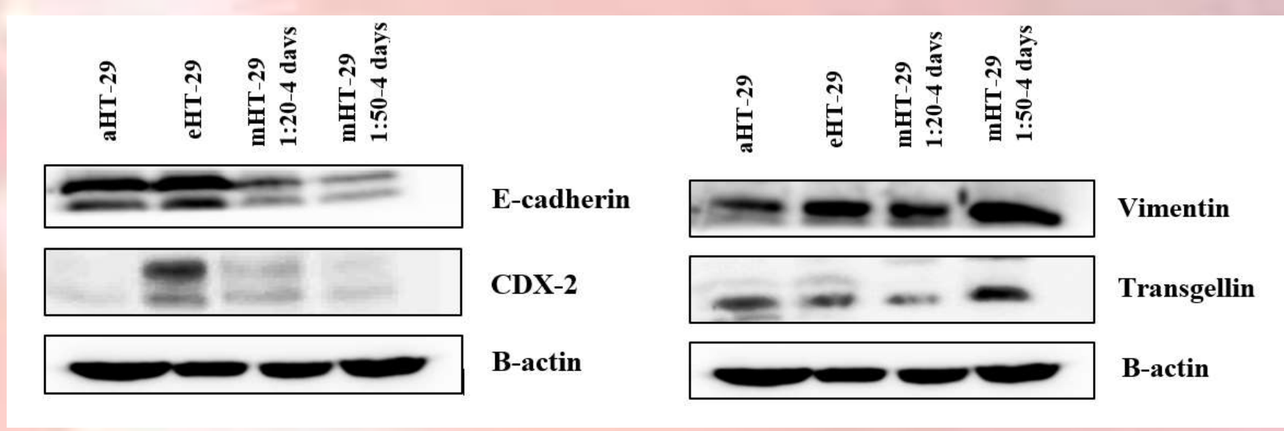
Total protein isolation from HT-29, eHT-29 and mHT-29 cells was performed in RIPA buffer containing protease inhibitor cocktail and PMSF. The Bradford method was used to measure the protein concentration of the cell lysate. Samples were prepared for WB analysis by mixing with 4X loading buffer. In Western blot analysis, samples were run on 12% SDS-PAGE gel and transferred to PVDF membrane. Then, the membrane was blocked and probed with appropriate antibodies according to the relevant protein. The chemiluminescent signal was detected in the substrate-treated bands.

REFERENCES:

1. Arlt, F., & Stein, U. (2009). Colon cancer metastasis: MACC1 and Met as metastatic pacemakers. *International Journal of Biochemistry and Cell Biology*, *41*(12), 2356–2359. https://doi.org/10.1016/j.biocel.2009.08.001
2. Koppens, M., & Van Lohuizen, M. (2016). Context-dependent actions of Polycomb repressors in cancer. *Oncogene*, *35*(11), 1341–1352. https://doi.org/10.1038/onc.2015.195
3. Tam, W. L., Weinberg, R. A. 2013. "The epigenetics of epithelial-mesenchymal plasticity in cancer." Nat Med 19 (11):1438-49.
4. Pattabiraman, D. R., Bierie, B., Kober, K. I., Thiru, P., Krall, J. A., Zill, C., Reinhardt, F., Tam, W. L., Weinberg, R. A. 2016. "Activation of PKA leads to mesenchymal-to-epithelial transition and loss of tumor-initiating ability." Science 351 (6277).
5. Wang, Y., Kuang, H., Xue, J., Liao, L., Yin, F., & Zhou, X. (2017). LncRNA AB073614 regulates proliferation and metastasis of colorectal cancer cells via the PI3K/AKT signaling pathway. *Biomedicine and Pharmacotherapy*, *93*, 1230–1237. https://doi.org/10.1016/j.biopha.2017.07.024

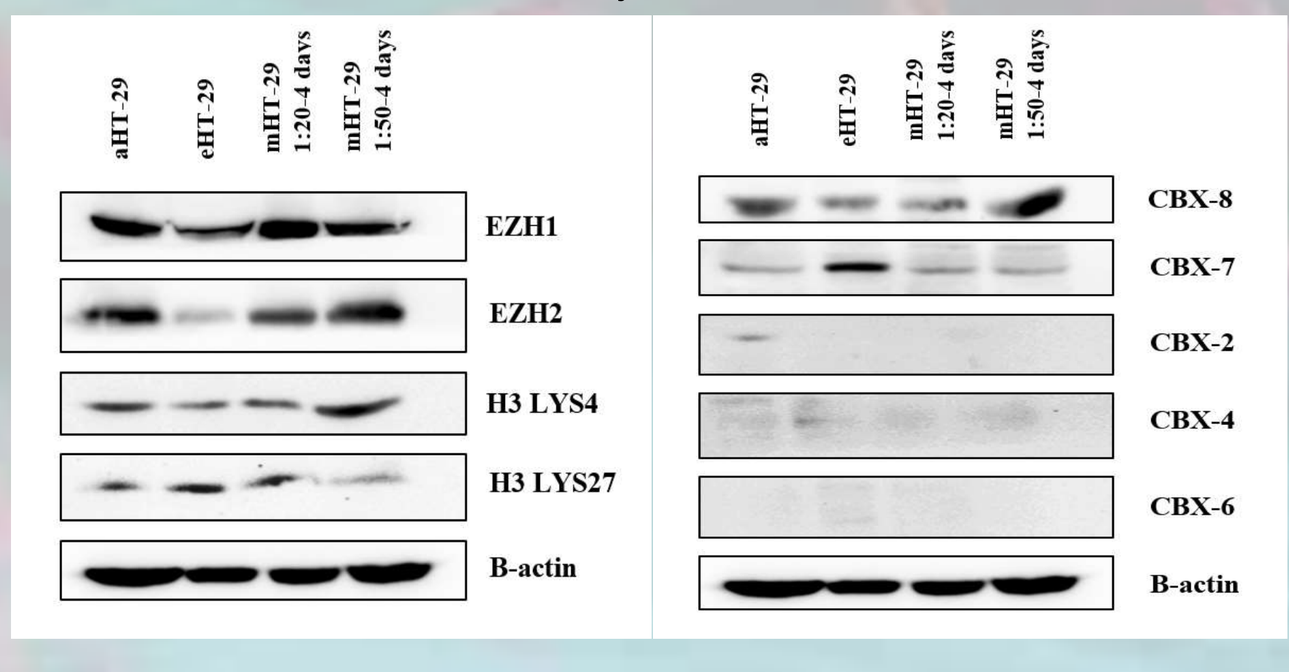
RESULTS AND DISCUSSION

1.Western blot analysis of epithelial (E-cadherin, CDX-2) and mesenchymal markers (Transgellin, vimentin) in HT-29 cells



The epithelial markers E-cadherin and CDX-2 increased in the samples on Day 20, which were expected to show epithelial characteristics, consistent with what was expected. An increase was observed in the amount of mesenchymal markers vimentin and transgellin in redifferentiation samples that were expected to show mesenchymal characteristics.

2.Analysis of the amounts of PRC2 member EZH paralogs (EZH1 and EZH2), PRC1 member CBX paralogs (CBX2, CBX4, CBX6, CBX7 and CBX8) and H3K27me3/H3K4me3 in HT-29 cells by western blot



According to the results we obtained; EZH1 and EZH2 were decreased in the epithelial differentiation group. In the epithelial differentiation group, H3 Lys4 decreased while H3 Lys27 increased. On the other hand, while H3 Lys4 increased in the redifferentiation group showing mesenchymal features, there was a decrease in H3 Lys27. An increase was observed in the differentiation group showing epithelial characteristics in CBX7. While CBX8 decreased in the differentiation group like H3 Lys4, it increased in the redifferentiation group.

CBX7 is the most studied CBX protein in the field of cancer. CBX7 is known to be downregulated in colon cancer. While CBX7 acts as an oncogenic in hematological malignancies, it acts as a tumor suppressor in epithelial-based malignancies. (Koppens & Van Lohuizen, 2016)

Upregulation of **EZH2** has been reported for many types of human malignancies, and increased EZH2 expression is generally associated with advanced tumor stage and poor prognosis. This is why EZH2 is often referred to as an oncogene. Recently, some mutations were found to increase EZH2 activity, while others inactivate the enzyme, suggesting that EZH2 may also act as a tumor suppressor. In colon cancer, however, EZH2 is down-regulated. (Koppens & Van Lohuizen, 2016) While EZH2 is up-regulated in many cancer types, it is down-regulated in colon cancer, making the investigation of EZH2 in colon cancer important.