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The Effects of Cytotoxic Dose of Diclofenac on CD82/KAI1 in Cervical Cancer Cells in Cell Culture

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***Abstract*:** *In this laboratory experimental study we assessed antimetastasis effects of Diclofenac on cervical cancer cells in cell culture. For this purpose, the cells (Hela cell line) were used in our study. MTT assay was used to determine cytotoxic effects of diclofenac followed by Real Time PCR to assay KAI gene expression in cells in which the extract have shown cytotoxic effects. Our results showed that administration of diclofenac (0.1 mg/ml) led to decrease in expression of antimetastasis KAI gene indicating "anti- antimetastasis" effects of diclofenac on cervical cancer cells in cell culture.*

Keywords: *Diclofenac, CD82/KAI1, ssssCervical Cancer*

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit the growth of several cancer cell lines. It concludes that aspirin and diclofenac inhibit the growth of fibroblast and cancer cell by inhibiting the up-regulation of cyclooxygenases enzymes in cancer cells. [1] The transmembrane protein CD82/KAI1 suppresses the metastatic potential of various cancer cell types. Moreover, decrease or loss of CD82 expression is closely associated with malignancy and poor prognosis in many human cancers including prostate cancer. Despite intense scrutiny, the mechanisms underlying the metastasis-suppressing role of CD82 are still not fully understood. Here, we found that a fibronectin matrix induced mesenchymal phenotypes in human prostate cancer cells with no or low CD82 expression levels. Altogether, these results suggest that CD82 suppresses EMT in prostate cancer cells adhered to the fibronectin matrix by repressing adhesion signaling through lateral interactions with the associated α3β1 and α5β1 integrins, leading to reduced cell migration and invasive capacities.[2]Carcinogenesis is a multifaceted intricate cellular mechanism of transformation of the normal functions of a cell into neoplastic alterations. Metastasis may result in failure of conventional treatment and death Hence, research on metastatic suppressors in cancer is a high priority. The metastatic suppressor gene CD82, also known as KAI1, is a member of the transmembrane 4 superfamily which was first identified in carcinoma of prostate. Little work has been done on this gene in breast cancer Our results suggest that lack of expression of the KAI1 might indicate a more aggressive form of breast cancer. Loss of KAI1 may be considered a significant prognostic marker in predicting metastatic manifestation. When evaluated along with the clinical and pathological factors, KAI1 expression may be beneficial to tailor aggressive therapeutic strategies for such patients. [3] Cervical cancer remains the leading cause of gynecologic cancer mortality orldwide. Unfortunately, neither improvements in surgical therapy nor modified radiotherapy methods have significantly decreased the mortality, and patients with advanced, recurrent

or metastatic disease still have a poor chance of cure. In this regard, promising novel treatment options such as molecular therapy strategies are needed. [4]

2. Material And Methods

Different concentrations of diclofenac (0.0001, 0.001, 0.01 and 0.1mg/ml) were prepared and used in our study. Hela cells (cervical cancer cell line) were purchased from National Cell Bank of Iran (Pasteur Institute, Tehran, Iran). Cells were grown and incubated in standard situation. Then, cells were sub-cultured into 75cm^2 flasks, 96-well plates or 6-well plates. Cytotoxicity of different doses of the extract was assayed using MTT method. To assess KAI gene expression we used Real Time PCR method. Analyses were conducted using the SPSS 20 and ANOVA.

3. Results

Our results showed that administration of cytotoxic dose (0.1 mg/ml) of diclofenac led to significant decrease in expression of KAI gene in Hela cells (Figure I).

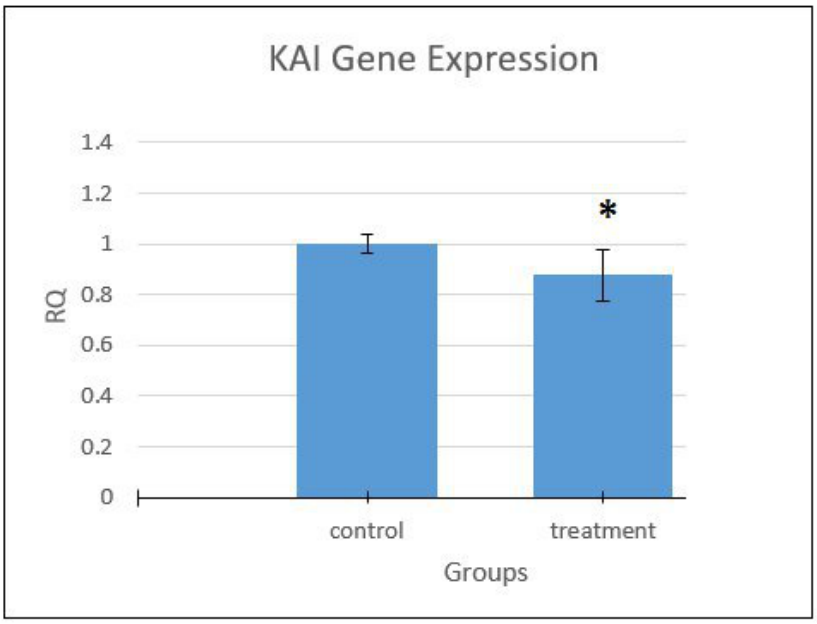

Fig. 1. Kai gene expression level in control and group exposed to diclofenac. * indicates significant increase compared to control group (P<0.01).

4. Discussion

We have found that diclofenac decreases Kai antimetastasis gene expression level. In line with our finding there are other studies indicating that diclofenac diminishes the expression of TERT, CDK4, CDK2, cyclin D1, and cyclin E genes. [5] However, cancer cells require nourishment for the growth of the primary tumor mass and spread of the metastatic colony. The inhibition of angiogenesis and induction of apoptosis by NSAIDs were found to be possible mechanisms in the chemoprevention of certain cancers. [6] Local drug release of anti-inflammatory agents should also be investigated as a therapeutic option in the prevention of tumor recurrence in oral squamous carcinoma. [7] Diclofenac has also been linked to increased risk of cardiovascular disease (CVD). [8] The recent identification of metastasis suppressor genes, uniquely responsible for negatively controlling cancer metastasis, are providing inroads into the molecular machinery involved in metastasis. While the normal function of a few of these genes is known; the molecular events associated with their loss that promotes tumor metastasis is largely not understood. KAI1/CD82, whose loss is associated with a wide variety of metastatic cancers, belongs to the tetraspanin family. [9], [10] Epidemiological studies have shown

that the regular use of non-steroidal anti-inflammatory (NSAIDs) drugs is associated with a reduced risk of various cancers. Previous studies also have shown that NSAIDs diclofenac exerts an anti-proliferative effect in ovarian cancer in vitro and in vivo and the effects of NSAIDs may be mediated, in part, by downregulation of E2F1. [11]

In contrast to our finding, further studies are warranted regarding the potential benefit of diclofenac and other NSAIDs to improve some types of cancers. [12] ,[13]

Cervical cancer is associated with abnormal expression of multiple genes. Survivin and Bcl-2 proteins are apoptosis inhibitors. The tumor suppressor gene CD82, which encodes the protein KAI1, is downregulated in cervical cancer, and is associated with differentiation degree. Overexpression of survivin and Bcl-2, and low expression of KAI1 promotes cervical cancer progress and metastasis. [14] The studies suggest that KAI1 may be used as a promising prognostic marker and a possible therapeutic target for human melanoma. [15] Decreased KAI1 expression has been observed recently in various human cancers, including pancreatic, lung, hepatic, colorectal, breast, ovarian, esophageal, and cervical cancers. Frequent down-regulation of the KAI1 protein was also observed in endometrial cancer cell lines. These data suggest that KAI1 expression is down-regulated in advanced endometrial cancer. Clinically it may be a useful indicator of the tumor progression and may provide prognostic information on the outcome of this disease. [16]-[18].

5. Conclusion

Our results showed that administration of diclofenac (0.1 mg/ml) led to decrease in expression of KAI gene, indicating "anti- antimetastasis" effects of diclofenac on cervical cancer cells in cell culture.

6. Acknowledgment

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7. References

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