DPP4/CD26 AS A MEDIATOR OF CHEMORESISTANCE AND EPITHELIAL-MESENCHYMAL TRANSITION IN HUMAN COLON CANCER CELLS

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Dipeptidyl peptidase 4 or Cluster of differentiation 26 (DPP4/CD26) is a multifunctional type II transmembrane glycoprotein, which regulates various biological processes *in vivo* and *in vitro* by cleaving N-terminal dipeptides with its extracellular domain [1]. Moreover, it can act as a receptor, transducing T cell costimulatory activation signal during interaction with different membrane proteins like ADA, CAV1, IGF2R, and PTPRC [2]. Also, DPP4/CD26 was shown to interact with extracellular matrix (ECM) proteins such as fibronectin and collagen, thus playing role in cellular processes as adhesion, migration and metastasis [3].

DPP4/CD26 has been intensively investigated as a potentional biomarker of various cancers and its impact on cancer malignancy and chemoresistance as well as a novel therapeutic target for different cancers during past decades. It can act as a tumor suppressor or activator depending on tumor type and some other conditions [3] [4]. It has been reported that subpopulation of $DPP4^+/CD26^+$ colon cancer cells showed elevated levels of epithelial–mesenchymal transition (EMT) protein markers in comparison with $DPP4^-/CD26^-$ subpopulation. DPP4/CD26 knock down in $DPP4^+/CD26^+$ subpopulation cells resulted in downregulation of EMT protein markers and upregulation of E-cadherin [5]. Also, DPP4/CD26 knock down was shown to downregulate some EMT markers in NLCSC cell lines *in vitro* [6]. In addition, vildagliptin, DPP4/CD26 enzymatic activity inhibitor, which is used to treat type 2 *diabetes mellitus* in clinical practice, contributed to elevated E-cadherin expression levels in MC38 cell line *in vitro* and suppressed colorectal lung metastases formation *in vivo* in mouse model [7]. However, in human breast cancer cell lines MCF 10A, MCF-7 and MDA-MB-231 DPP4/CD26 acts as an EMT negative regulator [8]. Moreover, this protein was proposed as a cancer stem cells marker, especially for colorectal cancer [5].

Colorectal cancer is the third in men and the second in women most common cancer worldwide, with nearly 1,8 million new cases diagnosed in 2018. Common therapy includes FOLFOX protocol, which consists of oxaliplatin (L-OHP) and 5-fluoruracil (5-FU) combination. However, colon tumor cells undergo fast adaptation and become resistant to anticancer agents. In addition, drug-resistant colorectal cell lines can demonstrate cancer stem cells-like properties, including DPP4/CD26 elevated expression [9]. However, it has not been investigated whether elevated DPP4/CD26 expression levels alone could cause development of chemoresistance or alter EMT status of colorectal cancer cells *in vitro*.

In this research, our proteomic analysis data showed, that colorectal cancer cell line HCT116, resistant to L-OHP (HCT-Oxa), exibited elevated DPP4/CD26 protein levels in comparison to parental HCT116 cell line (HCT-P), while HCT116 cell line, resistant to 5-FU (HCT-Fu), demonstrated comparable to HCT-P cell line DPP4/CD26 levels. Results were confirmed by western blot. Therefore, we checked if vildagliptin could alter resistance to L-OHP and 5-FU in HCT-P, HCT-Oxa and HCT-Fu cell lines. Also, vildagliptin influence on EMT status in these cell lines was investigated. After that, *DPP4* gene was amplified by polymerase chain reaction (PCR), using HCT-P cell line copy DNA (cDNA), cloned into pJET vector and sequenced. Consequently, *DPP4* sequence was transferred to pcDNA3 vector and this construct was used to investigate if transiently overexpressed DPP4/CD26 alters resistance to anticancer drugs in HCT-P and HCT-Oxa cell lines. Our next step was to clone *DPP4* sequence in all-in-one TET ON system lentiviral vector. Stable HCT-P and HCT-Oxa cell lines with an option of inducible DPP4/CD26 expression (HCT-P iDPP4, HCT-Oxa iDPP4) were created. Titration of DPP4/CD26 expression levels depending on doxycycline concentrations in these cell lines was completed. Finally, we investigated influence of maximum DPP4/CD26 expression level on chemoresistance and EMT status in HCT-P iDPP4 cell line.

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