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# DPPIV inhibitors extend GLP-2 mediated tumour promoting effects on intestinal cancer cells

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**Abstract**

*Background:* The glucagon-like peptides-1 and -2 (GLP-1 and -2) are co-secreted after food intake from intestinal L cells. Since both peptides are rapidly degraded by dipeptidyl peptidase-IV (DPPIV), research is focused on the development of DPPIV inhibitors or DPPIV resistant.
*Aims:* In this study we investigated, whether the inhibition of DPPIV activity and the resulting increased half-life of DPPIV substrates may influence cancer development and progression.
*Methods:* We examined proliferation and migratory activity of two human colon cancer cell lines (SW480, HT29) after stimulation with GLP-2 in combination with or without DPPIV inhibitors.
*Results:* Migratory activity was increased by 25% from 20% matrix induced activity to a maximum of 45% (100 nM GLP-2). In cells expressing CD26, migration was prolonged by addition of DPPIV inhibitors in a concentration dependent manner. After treatment with GLP-2 doubling time decreased from 2.4 to 1.5 days — and addition of DPPIV inhibitors enhanced the effect of GLP-2.
*Conclusions:* The use of DPPIV inhibitors together with GLP-2 led to increased proliferation as well as elevated migratory activity. Therefore, the use of DPPIV inhibitors could increase the risk of promoting an already existing intestinal tumour and may support the potential of colon cancer cells to metastasize.


*Keywords:* Glucagon-like peptide; Dipeptidyl peptidase-IV; Proliferation; Migration

## 1. Introduction

Over the past few years increasing evidence was found in epidemiological studies that obese people, diabetic patients and/or people with elevated glucose serum levels have a higher incidence of developing cancer at several organ sites, including colon [1]. In part, this elevated cancer risk may be explained by alterations in the metabolism of endogenous hormones (e.g. sex steroids, insulin, and insulin-like growth factors), which can lead to distortion of normal balance between cell proliferation, differentiation, and apoptosis [2].

The Glucagon-like peptide-2 (GLP-2) is an intestinal peptide hormone derived from the tissue-specific, post-translational processing of the proglucagon gene [3]. After nutritional intake, GLP-1 and GLP-2 are secreted from intestinal enteroendocrine L cells. While GLP-1 is leading to an increased secretion of insulin [4], GLP-2 causes increased intestinal hexose transport respectively [5]. Once released, GLP-2 regulates gastric motility, gastric acid secretion, intestinal hexose transport, increases crypt cell proliferation and regulates inhibition of apoptosis in the enterocyte and crypt compartments [6]. GLP-2 also enhances intestinal epithelial barrier function by affecting both paracellular and transcellular pathways [7]. The signals of GLP-2 are mediated through the GLP-2 receptor (GLP-2R), a subclass B receptor of G protein-coupled receptors which is expressed in cells of the stomach, jejunum, ileum, and colon [8], but is also found in the brain [3].

The biological activities of GLP-1 and GLP-2 are regulated by the proteolytic cleavage of the first two N-terminal amino acids by dipeptidyl peptidase-IV (DPPIV) [9,10]. DPPIV – also known as CD26 – is a cell surface glycoprotein that serves in signal transduction and as a proteolytic enzyme [11]. First discovered in immune cells, CD26 functions as a co-stimulatory molecule in lymphocytes, where its expression is regulated upon the activation of the cells. In contrast, CD26 is constitutively

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expressed on epithelial cells of the intestine, kidney and liver — and also as a soluble form ubiquitously found in serum [12]. Given that the post-proline cleavage site is found ubiquitously, CD26/DPPIV has a wide range of substrates, which can either be activated by DPPIV cleavage (e.g. IL-2) or deactivated (e.g. GLP-1, GLP-2). Because of the deactivating function, the development of DPPIV resistant peptides or DPPIV inhibitors came into the focus of various studies [13,14]. Especially the application of GLP-1 as a possible treatment of type II diabetes, but also GLP-2 to treat inflammatory bowel syndrome would benefit from the development of DPPIV inhibitors. Also the treatment of autoimmune diseases could potentially profit from such a progression [15].

Here we report on possible side effects of the usage of DPPIV inhibitors besides their already established functions such as the promotion of lowering blood glucose levels [16] or the reconstitution of intestinal tissue [17]. Focusing on the well established functions of GLP-2 on intestinal cells under physiological conditions, we investigated GLP-2 mediated effects on cell proliferation and migration and determined its activities on colon carcinoma cell lines of different tumour stages.

## 2. Materials and methods

### 2.1. Cell culture

The well differentiated (grade one) human colon carcinoma cell line HT29 (obtained from the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany)) was incubated in McCoy's 5A (PAA Laboratories GmbH, Linz, Austria) supplemented with 10% bovine serum and 1% pen/strep in a humidified atmosphere with 5% $CO_2$ addition. The poorly differentiated (grades III–IV) SW480 cell line (obtained from American Type Culture Collection (ATCC, Rockville, MD)) was grown in antibiotic-free Leibovitz L-15 medium (PAA Laboratories GmbH, Linz, Austria), supplemented with 10% FCS in a humidified atmosphere without $CO_2$ addition.

### 2.2. Flow cytometry

Flow cytometry was used to determine GLP-2R and CD26 expression. We incubated $1 \times 10^5$ cells with 10 μg/ml of the primary antibody (rabbit anti-GLP-2R (GLP-2R11A, Alpha Diagnostic International, TX, USA/Biotrend, Germany)) or mouse anti-CD26 (clone BA5, Santa Cruz, Ca) overnight at 4 °C, and subsequently washed the cells three-times with PBS. For detection, a 1 μg/ml solution of a FITC-conjugated secondary goat anti-rabbit, or goat anti-mouse antibody, was used, incubated at 4 °C overnight, followed by three washing steps with PBS. To determine unspecific binding, an anti-IgG antibody was used as isotype in combination with the same FITC-labeled antibodies.

### 2.3. Immunoblotting

To confirm results of flow cytometry, we used 500,000 colon cancer cells per sample lysed in Laemmli sample buffer and incubated for 10 min at 95 °C. Approximately 200 mg of mouse small intestine and colon served as positive controls for GLP-2R detection. Mouse tissue was lysed by rapidly freezing in liquid nitrogen and thawing, followed by incubation in Laemmli sample buffer for 10 min at 95 °C. Proteins were separated via SDS-PAGE and transferred to an Immobilion-P membrane (Millipore, Bedford, MA) followed by one hour blocking with 5% milk powder in PBS-Tween at room temperature. After overnight incubation at 4 °C with the primary antibody (rabbit anti-GLP-2R (GLP-2R11A, Alpha Diagnostic International, TX, USA/Biotrend, Germany)), the membrane was washed vigorously with PBS-Tween. Subsequently, the membrane was first incubated with a peroxidase-linked anti-mouse antibody (1 μg/ml, 2 h, 20 °C) and then with a chemiluminescence substrate (90 s, 20 °C; Boehringer Mannheim). The chemiluminescence signal was detected by a Hamamatsu digital camera type C4742-98.

### 2.4. Analysis of cell movement

Cell migration assays were performed as described previously [18,19]. In brief, a liquid collagen solution was mixed with minimal essential medium, sodium bicarbonate, tumour cells, and substances to be tested. The collagen gels were filed into self-constructed chambers and were allowed to polymerize for 20 min at 37 °C. The chambers were then filled up with serum-free medium containing all components in the same concentration as in the collagen lattices and sealed with wax. Tumour cell locomotion within the 3D collagen lattices was recorded by time lapse video microscopy. For data analysis, 30 cells of each sample were randomly selected and projections of the paths were digitized as $x/y$ coordinates in 15-minute intervals by computer-assisted cell tracking. Data are displayed either as average lines of 15-minute intervals of 90 cells or as bar graphs summarizing the average of the activity of all 15-minute intervals.

### 2.5. Data analysis

Statistical comparison between specified values was made either by ANOVA using a Dunnett's multiple comparisons test or the Bonferroni multiple comparisons test.

### 2.6. Proliferation assay

On day zero $1 \times 10^5$ cells were seeded into 24-well plates and incubated under normal culture conditions (control), or incubated with either GLP-2 alone or in combination with a DPPIV inhibitor, respectively. Each day, two wells per sample were trypsinized and cells were counted to establish cell growth curves. In the remaining wells, the medium was replaced by fresh medium with the appropriate supplements. Cell growth curves were taken from each cell line at various conditions. To determine proliferation rates, exponential phases of each cell growth curve were used for calculations. A MTT proliferation assay (CellTiter 96 Aqueous One Solution Cell Proliferation Assay/Promega, Madison, WI, USA) was performed according to the manual, to confirm data received from cell growth curves.