International Doctoral School
Medical University of Lodz

Diamentowy Grant

Cathelicidin LL-37 and its derivatives in therapy of colorectal cancer

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Introduction

Colorectal cancer (CRC) is one of the leading causes of morbidity and mortality worldwide. Worldwide, the number of new cases is approximately 1.36 million per year. Moreover, further increase in the number of new cases, including people under 50 years of age, is expected. Current CRC therapies based on surgical resection and 5-fluorouracil chemotherapy are not fully satisfactory, therefore new, more effective therapeutic interventions are necessary.

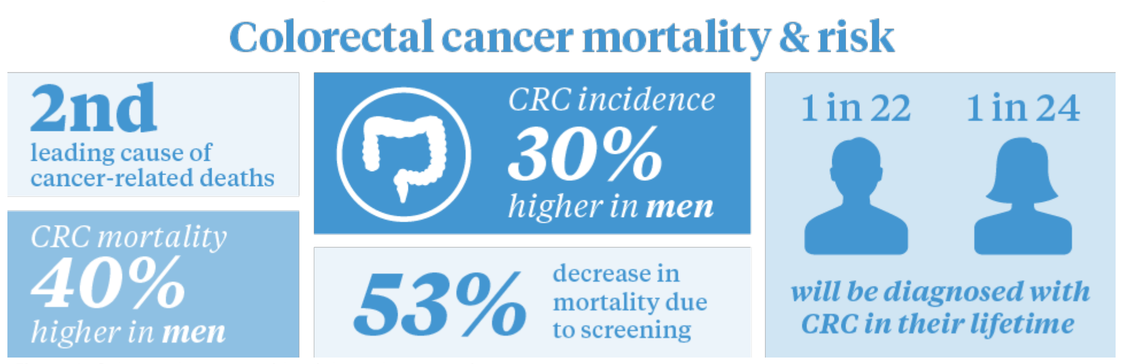

Figure 1. Colorectal cancer epidemiology characterstics.

The group of **antimicrobial peptides** includes cathelicidins and defensins, linked with a wide range of activities in the immune response to numerous pathogens. Research on cathelicidin LL-37 suggests its participation in inflammation and the process of carcinogenesis.

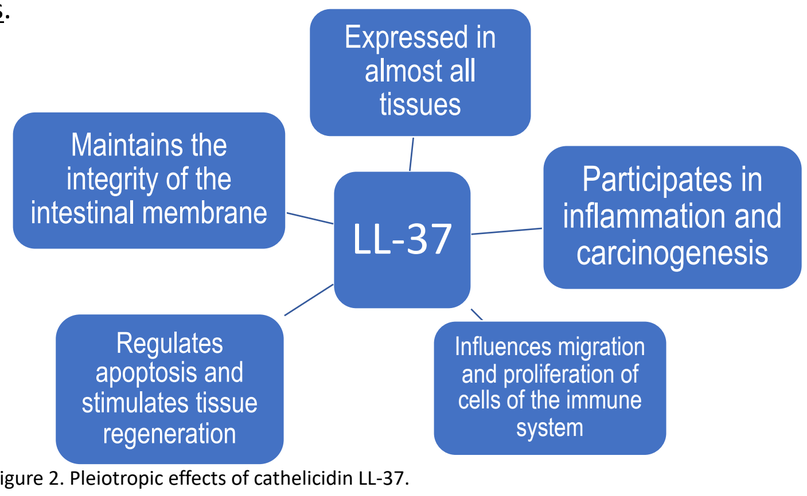

Figure 2. Pleiotropic effects of cathelicidin LL-37.

AIM AND RESEARCH HYPOTHESIS

The aim of the project is to understand the mechanism of action of the antimicrobial peptide LL37 and to try to use its derivatives as a new, potential anticancer therapy.
In the project I will try to answer the question whether derivatives of the biologically active fragment of cathelicidin LL37, the KR-12 peptide, modified with short fatty acids, can inhibit the carcinogenesis process in the large intestine.

Methodology

In vitro studies and in vivo model

- Synthesis of compounds (KR-12 peptide and its derivatives modified with short-chain fatty acids).
- Assessment of the influence of tested compounds on viability of colorectal cancer and normal colon epithelium cell lines.
- Evaluation of the action of selected compounds in a mouse model of colorectal cancer.
- Molecular and histopathological analysis of the material obtained from the animal model.

Studies on the mechanism of action of the LL-37 peptide in human tissue and its dependence on vitamin D

- Collection of human colorectal cancer tissue
- Molecular analysis of the obtained tissues.

Sex	Male	N=10
	Female	N=10
Age		55.46 ± 12.37
Size [cm]		5.86 ± 2.41
Localization	Rectum	N=7
	Sigmoid	N=4
	Descending colon	N=2
	Transverse colon	N=3
	Ascending colon	N=4
Nodes	N0	N=10
	Nx	N=10
T stage	T2	N=5
	T3	N=12
	T4	N=3
Grade	1	N=6
	2	N=10
	3	N=4

Table 1. Characterstics of the colorectal cancer patients qualified to the study.

Results

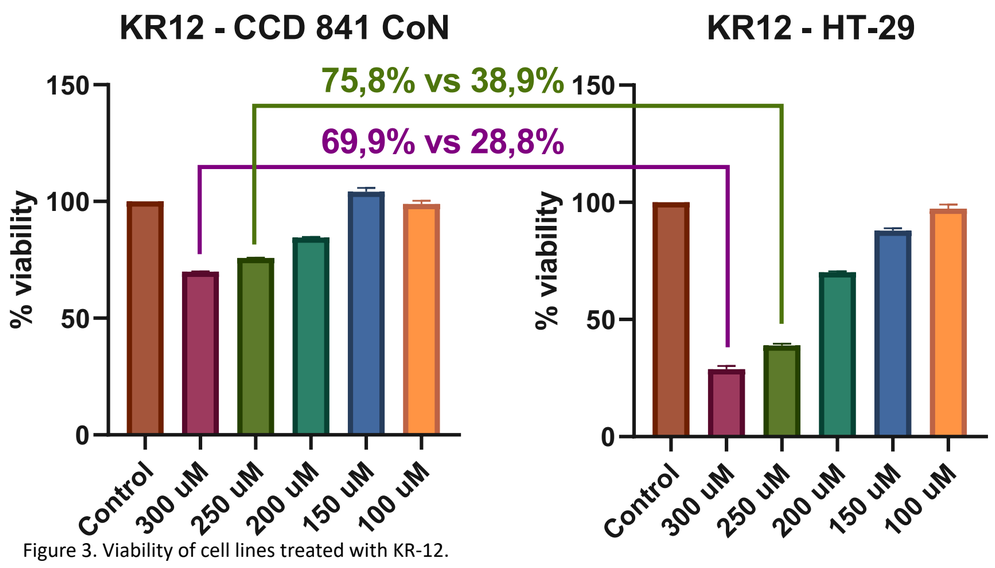

Figure 3. Viability of cell lines treated with KR-12.

- KR-12 at a concentration of 300 µM significantly reduced the viability of HT-29 cells (28.8%), while at this concentration, the viability of CoN cells was 70.1%. (Fig.3)
- KR-12 modified with acetic acid at a concentration of 300 µM decreased the viability of HT-29 and CoN cells in a similar range (52.5% and 61.9%, respectively).
- KR-12 modified with butyric acid at a concentration of 300 µM also decreased the viability of HT-29 and CoN cells to a similar extent (42.8% and 44.9%, respectively)
- KR-12 modified with 300 µM propionic acid significantly decreased the viability of colon cancer cells (52.3%). However, the same concentration increased the viability of CoN cells to 100.2%. (Fig.4)
- LL-37 and FPR2-2 expression levels were significantly increased in the tumor tissue compared to tissue from the healthy margin (Fig.5)
- TLR3 expression was significantly reduced in the tumor tissue compared to tissue from the healthy margin (Fig.5)

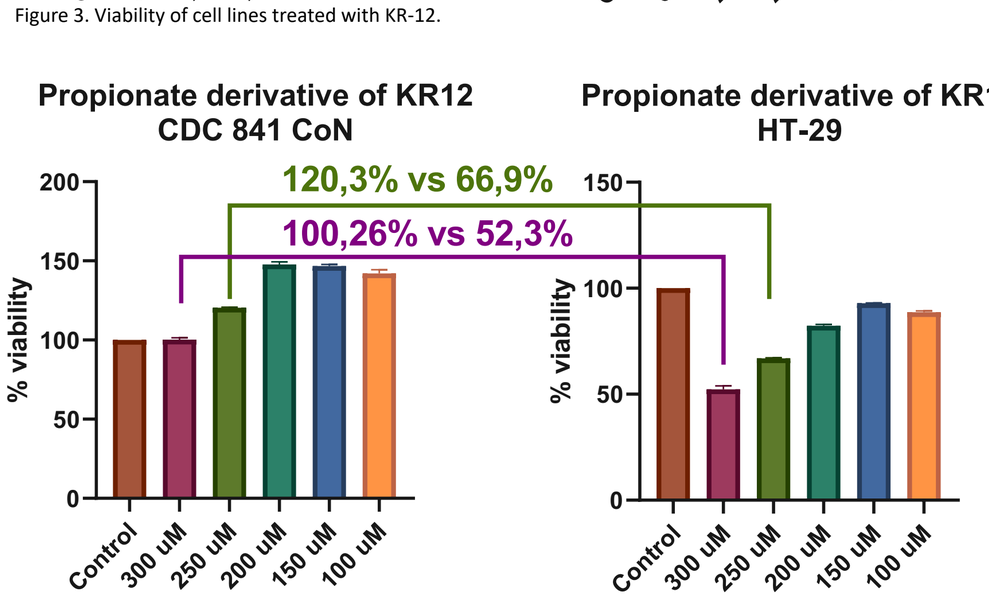

Figure 4. Viability of cell lines treated with KR-12 modified with propionic acid.

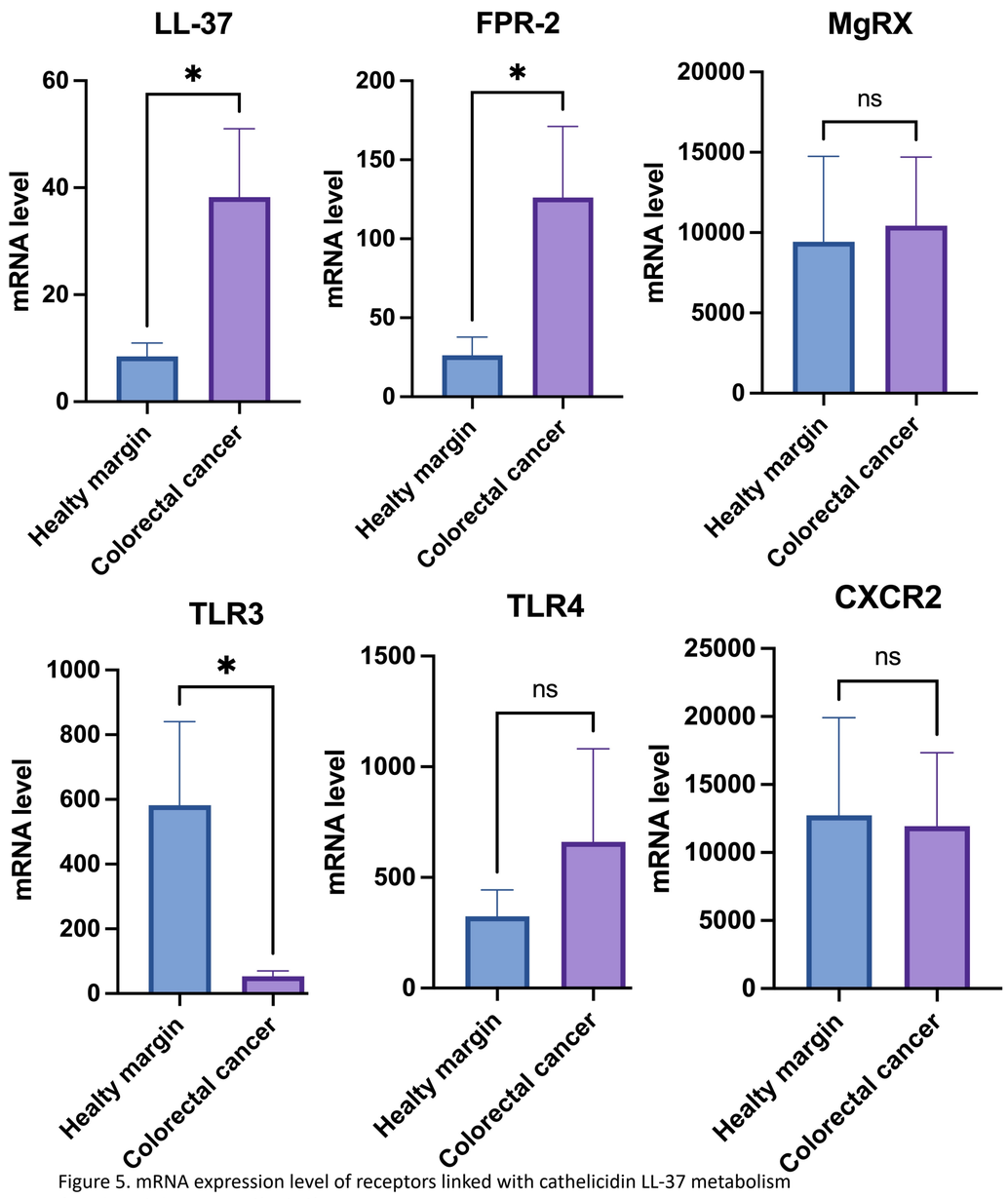

Figure 5. mRNA expression level of receptors linked with cathelicidin LL-37 metabolism in colorectal cancer patients tissue.

Conclusions

KR-12 compounds modified with short-chain fatty acids show promising anti-cancer properties. KR-12 and KR-12 modified with propionic acid decreased the viability of cancer cells most effectively, while at the same time they were not toxic to normal cells. The anti-tumor effect of these compounds will be confirmed by evaluating the apoptotic protein panel of these cell lines.
Cathelicidin LL-37 expression is significantly elevated in colorectal cancer tissues compared to healthy intestinal epithelium. Potential receptors involved in cathelicidin action are TLR3 and FPR-2.