

IMMUNOHISTOCHEMICAL EXPRESSION OF VITAMIN D RECEPTOR IN DEVELOPMENT STAGES OF COLORECTAL CARCINOMA

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SUMMARY

Background: Chemo preventive and antitumor role of vitamin D is manifested through genetic and non genetic ways with a powerful antiproliferatory and proapoptotic effect, which is proven by numerous epidemiologic studies. The genetic activity of vitamin D is determined through vitamin D receptors (VDR), a member of stero-thyreoidal family of nuclear receptors, which with vitamin D form a cell nucleus complex responsible for the chemo preventive and antitumor effect.

VDR in tissue cells is present in the cytoplasm and the nucleus and manifests its genetic activity after transfer from the cytoplasm to the nucleus. The mechanisms for the transport and genetic control of the transport of VDR from cytoplasm to the nucleus in not yet completely understood.

Subjects and methods: By using immunohistochemistry we are evaluating the correlation of cytoplasmic and nuclear expression of VDR during different stages of colorectal carcinoma: normal colorectal mucosa, hyperplastic polyp, low grade adenoma (LGD), high grade adenoma (HGD) and colorectal cancer.

Results: Our results confirm that the nuclear VDR expression is strongest in normal colorectal mucosa and in hyper plastic polyps, is gradually weakened in low and high grade adenoma while it is extremely weak or absent in colorectal carcinoma. At the same time the expression of cytoplasm VDR is weakest in normal colorectal mucosa and hyper plastic polyps while it grows during the adenoma stage and is most expressed during colorectal carcinoma

Conclusion: We conclude that vitamin D has a strong chemo preventive and antitumor effect in normal colorectal mucosa and hyper plastic polyps, while its antitumor and chemopreventive effect is progressively weakened and ultimately absent in colorectal carcinoma.

Key words: vitamin D receptors (VDR) - colorectal mucosa - hyper plastic polyp-colorectal adenoma-colorectal carcinoma (CRC)-expression

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INTRODUCTION

Vitamin D is a steroid hormone with proven chemo preventive and antitumor effects in different types of tumors including CRC (Giovannucci 2005, Pitz et al. 2009). Direct connection with serum concentration of vitamin D, CRC risk and survival is proven with clinical studies (Garland et al. 1989, Rheem et al. 2010).

Chemopreventive and antitumor effects of vitamin D are manifested in genetic and non genetic way, through inhibition of cell proliferation, neoangiogenesis and through stimulating apoptosis and cell differentiation (Moreno et al. 2005, Iseki et al. 1998, Palmer et al. 2001, Diaz et al. 2000).

Non genetic way of vitamin D effects is manifested through calcium metabolism and genetic way through VDR, a highly specific transcription and transactivation

factor which modulates gene expression of transport protein included in calcium metabolism and whose expression directly determines the gene effects of vitamin D, meaning its chemopreventive and antitumor effect (Ali et al. 2009).

Research has shown the 25% of circulating vitamin D is bonded to VDR after which gene mechanisms of reduced proliferation and hastened apoptosis are manifested in colorectal carcinogenesis (Hendrickson et al. 2011).

VDR expression of the cytoplasmic and nuclear VDR expression ration in different tumors in determined by histopathological characteristics of the tumors, tumor development stage and differentiation grade of the tumors.

Research in cytoplasmic and nuclear ratio of expression in healthy bronchial mucosa and in dysplastic mucosa

and bronchial carcinoma has shown that cytoplasmic VDR expression is gradually weakened while nuclear expression is present continuously. That suggests the possibility of vitamin D use as a chemopreventive agent in bronchial carcinoma (Menezes et al. 2008).

Research in the connection of VDR expression and macrocellular bronchial carcinoma survival showed only nuclear expression of VDR as a prediction factor for survival, while cytoplasmic VDR expression had no value as a prediction factor (Srinivasan et al 2012).

VDR expression in colorectal carcinoma has been examined in multiple studies with conflicting results.

Study done on biological material concluded that VDR is most expressed in normal colorectal mucosa and gradually wakens towards the colorectal carcinoma stage (Palmer et al. 2004). Other studies show lowered VDR expression only in adenocarcinoma stage with lack of expression present in lymph node metastasis or other organ metastasis (Sheinin et al. 2000). Other authors find low expression in normal colorectal mucosa in their studies. In those studies VDR expression grows with colorectal carcinoma development (Matusiak et al. 2005).

SUBJECTS AND METHODS

VDR expression was retrospectively analyzed in representative paraffin bloc tissue samples after colorectal surgery, which corresponds to histological stages of colorectal mucosa. 5 groups of 20 samples were formed for this research:

- Normal colorectal mucosa;
- Hyper plastic polyp;
- Low grade dysplasia adenoma;
- High grade dysplasia polyp;
- Colorectal carcinoma.

VDR expression samples were obtained by 3-5 μ tissue cuts from archived paraffin block. Microscopic analysis was done with Olympus BX41 microscope. Each sample was analyzed in 10 fields of view of high magnification. The counting of positive cells was done with Cell D1 Image Analysis program (Olympus).

We used mouse monoclonal antibody for VDR expression (clone VDR, D-6, sc-13133, Biotechnology, Santa Cruz, USA) 1:100 dilution.

We used quantitative method of establishing color intensity in samples which has 4 stages (Kure et al. 2009).

- 0 – No expression
- 1 – Weak expression
- 2 – Moderate expression
- 3 – Strong expression

Statistical analysis

We used descriptive statistics to show distribution of analyzed data and Fisher's Exact test to show strength of expression in different stages of colorectal cancer development.

RESULTS

According to our results nuclear expression of VDR is strongest in normal colorectal mucosa and hyperplastic polyps. In LGD adenoma stage and HGD adenoma stage expression of VDR is weaker and is the weakest in CRC stage. There is statistical significant difference between different stages (Table 1).

In normal colorectal mucosa and hyperplastic polyps cytoplasmic expression of VDR is weakest, becomes gradually stronger in LDG and HGD adenoma stages and is strongest in CRC. There is statistically significant difference between stages (Table 2).

Table 1. The expression of nuclear vitamin D receptor in the developmental stages of colorectal cancer

VDR/N	Group N (%)				
	Normal mucosa	Hiperplastic polypus	Adenoma - LGD	Adenoma - HGD	CRC
Negative	0 (0)	0 (0)	1 (5)	11 (55)	17 (85)
Weak positive	0 (0)	0 (0)	5 (25)	2 (10)	1 (5)
Moderately positive	18 (90)	16 (80)	13 (65)	7 (35)	2 (10)
Very positive	2 (10)	4 (20)	1 (5)	0 (0)	0 (0)

LGD - low grade dysplasia; HGD - high grade dysplasia; VDR/N - nuclear vitamin D receptor; Fisher's Exact Test = 71.87; p<0.001

Table 2. The expression of cytoplasmic vitamin D receptor in the developmental stages of colorectal cancer

VDR/N	Group N (%)				
	Normal mucosa	Hiperplastic polypus	Adenoma - LGD	Adenoma - HGD	CRC
Negative	5 (25)	4 (20)	0 (0)	0 (0)	2 (10)
Weak positive	15 (75)	15 (75)	11 (55)	6 (30)	5 (25)
Moderately positive	0 (0)	1 (5)	6 (30)	5 (25)	4 (20)
Very positive	0 (0)	0 (0)	3 (15)	9 (45)	9 (45)

LGD - low grade dysplasia; HGD - high grade dysplasia; VDR/C - cytoplasmic vitamin D receptor; Fisher's Exact Test = 46.67; p<0.001

DISCUSSION

We analyzed cytoplasmic and nuclear VDR expression in CRC development stages (normal colorectal mucosa, hyperplastic polyp, LGD adenoma, HGD adenoma and CRC). The results were compared to previous research.

According to obtained results the expression ratio of VDR/C and VDR/N changes with histological grade of colorectal mucosa.

Nuclear VDR expression is strongest in normal colorectal mucosa and hyperplastic polyps while cytoplasmic expression is minimal or absent in these stages.

As the genetic effect of VDR requires VDR expression we conclude that the chemoprevention and antitumor effects of vitamin D are strongest in normal colorectal mucosa and hyperplastic polyp.

In LGD and HGD adenoma nuclear VDR expression is weak compared to a healthy colorectal mucosa and hyperplastic polyp, which in turn means that the genetic effect of vitamin D is weak as well. Genetic effects of vitamin D are weakest in CRC as the nuclear VDR expression is almost completely absent.

Weakened nuclear VDR expression is accompanied with stronger cytoplasmic VDR expression which plays no role in genetic effect of VDR.

Based on the obtained results we conclude that certain gene mechanism important to cytoplasmic VDR transport to the nucleus are being blocked as early as LGD and HGD adenoma stage. That leads to retention of VDR in the cytoplasm and gradual weakening of VDR expression

Gene mechanisms which coordinate VDR transport from cytoplasm to the nucleus are completely blocked in CRC as the nuclear VDR expression is weakest in that stage.

We conclude that nuclear VDR expression can be an important prediction marker and could have important predictive significance in treating colorectal cancer.

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Contribution of individual authors:

All authors contributed to the conception of the article. First draft was done by Nikica Šutalo and Snježana Tomić. Acquisition of data and surgical and oncology treatment analysis of small bowel carcinoma in this paper has been done by Vedran Dragišić and Inga

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