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Vitamin D receptor and cellular retinol-binding protein-1 immunohistochemical expression in normal, hyperplastic and neoplastic endometrium: Possible diagnostic and therapeutic implications☆

Dalia M. Badary[a,*], Hisham Abou-Taleb[b]

[a] Pathology Department, Faculty of Medicine, Assiut University, Egypt.
[b] Department of Obstetrics and Gynecology, Faculty of Medicine, Assiut University, Egypt

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ABSTRACT

Background: We conducted this study to assess the effect of VDR and CRBP-1 immunohistochemical expression on the endometrium and to explore their role in endometrial cancer carcinogenesis.
Methods: This study comprised two hundred paraffin-embedded endometrial tissue samples diagnosed as 42 and 63 proliferative and secretory endometrium respectively, 45 endometrial hyperplasias with atypia and 50 endometrial carcinomas (25 low-grade and 25 high-grade endometrial carcinomas). The immunohistochemical method was done to determine the expression of VDR and CRBP-1.
Results: VDR was strongly expressed in 8 (17.8%) cases with endometrial hyperplasia, 15 (60%) cases with low-grade endometrial carcinoma, and 22 (88%) cases with high-grade endometrial carcinoma. While CRPB1 overexpression was noted in cases with proliferative endometrium, secretory endometrium and endometrial hyperplasia with atypia, 37 (88.1%), 56 (88.9%) and 3 (6.7%) cases respectively and all malignant cases showed negative expression.
Conclusions: Increased VDR expression and reduced CRBP-1 expression are associated with malignant features of the endometrium with a significant statistical difference of immunoreactivity between groups of normal endometrium, hyperplastic changes & carcinoma. Our data suggested that increased VDR expression is partly associated with endometrial cancers through a premalignant phase. Also, increased VDR and reduced CRBP-1 expression are associated with the progression of endometrial carcinoma with higher grades.

1. Introduction

In the last decade, vitamin D has gained more significance when it was shown to affect various medical problems like diabetes, cardiovascular disease, and cancer [1].

Vitamin D is obtained through endogenous synthesis and diet; the endogenous synthesis is the primary source [2], vitamin D synthesis is dependent on UVB radiation. It starts with the oxidation of cholesterol to 7-dehydrocholesterol in the bowel epithelium then transported and converted in the skin to pre-vitamin D3 by ultraviolet radiation. At dependent reaction temperature, pre-vitamin D3 isomerizes to vitamin D3 which activated to 1α,25 (OH)2D3 (calcitriol) by the mitochondrial and microsomal vitamin D 25-hydroxylases in the liver and the renal mitochondrial 1-hydroxylase [3]. Calcitriol synthesis can be found in organs rather than the kidney as skin, prostate, and cancer cells and exercises his functions on tissues by binding to the vitamin D receptor (VDR) [4,5]. The VDR has been found in 30 different tissues and numerous studies have shown VDR's role as a mediator in inflammation, estrogen-related pathways, and insulin-like growth factor signaling [6]. Also, the VDR is expressed in many tumor tissues, indicating that it influences cancer etiology [7]. There are large numbers of epidemiological and preclinical studies that were done to know the impact of vitamin D and its receptor on cancer progression and mortality [8]. Many studies showed that high circulating levels of vitamin D are accompanied by a decreased risk of developing certain types of cancer as breast, hematological, colorectal, gastric kidney, head, and neck, liver,

Abbreviations: VD, Vitamin D; VDR, Vitamin D receptor; CRBP-1, Cellular retinol-binding protein-1; FIGO, The International Federation of Gynecology and Obstetrics; IRS, Immunoreactive score

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* Corresponding author at: Pathology Department, Assiut University, Al-Gamaa Street, Assiut 71526, Egypt.
E-mail addresses: Daliabadary@gmail.com, daliabadary@aun.edu.eg (D.M. Badary).

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pancreatic, lung, ovarian, and skin cancer [9]. It has been shown that vitamin D induces differentiation and inhibits the proliferation of cancer cells in vitro and in vivo [9]. A very limited number of studies analyzing vitamin D and its receptor role in endometrium and its influence on endometrial cancer occur.

Retinol and vitamin A derivatives affect cell differentiation, proliferation, and apoptosis and play an essential role in numerous biological processes [10]. Retinol is obtained from animal foods. Retinol derivatives are crucial for vision, while retinoic acid is fundamental for skin and bone growth [11]. Intracellular retinoid bioavailability is controlled by specific cytoplasmic retinol and retinoic acid-binding proteins (CRBPs and CRABPs). CRBP-1, the most diffuse CRBP isoform CRBP-1 assumes the main role in arterial tissue remodeling processes and wound healing [12]. In the most recent years, the job of CRBP-1-related retinoid signaling during cancer progression became the object of numerous studies. CRBP-1 gene function in controlling the availability of retinol to cells suggests that its product has a role in the inhibition of early steps in transformation [13]. CRBP-1 is down-regulated in ovarian and breast tumors and compromises RAR activity, causing loss of cellular differentiation and tumor progression [14]. Also, the CpG island hyper-methylation of CRBP-1 causing its inactivation in some cancer cell lines as larynx, nasopharyngeal, cervix, lymphoma, and gastrointestinal carcinomas [15]. So, losing CRBP-1 expression is common in many human cancers that may have a prevention role in cancer occurrence and new therapeutic strategies using retinoids [13]. Few studies on CRBP-1 expression in the endometrium, including its cancer.

Hence, we conducted this study to assess the effect of VDR and the CRBP-1 expression on the endometrium (proliferative, secretory, hyperplastic with atypia, low grade, and high-grade endometrial carcinomas) and to explore their role in endometrial cancer carcinogenesis via their immunohistochemical expression.

2. Materials and methods

2.1. Materials

In this descriptive-analytic study, our samples comprised two hundred paraffin-embedded endometrial tissue samples diagnosed as normal endometrium consisting of 42 and 63 proliferative and secretory endometrium respectively, 45 endometrial hyperplasia with atypia and 50 endometrial carcinomas (25 low-grade endometrial carcinomas including endometrioid adenocarcinoma FIGO 1 and 2 and 25 high-grade endometrial carcinoma including endometrioid adenocarcinoma FIGO 3, serous adenocarcinoma, clear cell adenocarcinoma, carcinosarcoma, and undifferentiated carcinoma). The samples were selected from surgical pathology files of pathology laboratory at Woman Health Hospital, Assiut University, Egypt. The selected samples in the present study were 137 curettage specimens and 63 hysterectomy specimens. The samples were obtained from patients (age range 20–80 years) and the Hematoxylin and eosin-stained slides for each case were reviewed by an expert pathologist to confirm the histopathological diagnosis. Specimens with any evidence of endometrial polyp, endometritis, and endometrial cancers other than endometrial carcinoma were excluded and the most representative paraffin block for each case was selected for immunohistochemical studies.

2.2. Immunohistochemical methodology

A panel of VDR and CRBP-1 proteins were analyzed by immunohistochemical staining using the avidin-biotin immunoperoxidase complex technique following the manufacturer's protocol. Tissue sections (4-μm thick) of formalin-fixed, paraffin-embedded specimens were cut and dried in a 60 °C oven overnight. Sections were deparaffinized in xylene, rehydrated in graded alcohol, and transferred to PBS (phosphate-buffered saline, PH 6). The sections placed in an endogenous peroxide block for 15 min and subsequently applied VDR (mouse monoclonal antibodies, Lab Vision Corporation, Fremont, CA, USA, 1: 100) and CRBP-1 (mouse monoclonal antibody, Lab Vision Corporation, Fremont, CA, USA, a dilution of 1: 100 for 60 min. The primary and secondary antibodies were applied for 10 min at room temperature, then immunocomplexes were visualized with diaminobenzidine for 10 min and covered by a coverslip. Finally, the slides were examined by Olympus light microscopy.

2.3. Immunohistochemical evaluation

The cytoplasmic immunohistochemical expression of CRBP-1 and nuclear immunohistochemical expression of VDR was evaluated semi-quantitatively according to the Remmele immunoreactive score (IRS) [16-18]. The intensity of the immunohistochemical reaction scaled from 0 to 3 and multiplied by the percentage of positively stained endometrial cells which was divided into five grades of 0–4 (0%, < 10%, 10–50%, 51–80% and > 80%). Then the obtained IRS was interpreted as 0 to 1 considered negative expression; 2 to 3 considered weak expression; 4 to 8 considered moderate expression and 9 to 12 considered strong expression.

2.4. Statistical analysis

We assessed the correlations between VDR and the CRBP-1 expressions and the histopathological diagnosis of the specimens using the chi-square test (Fisher's exact test). We examined correlations between their expressions using the chi-square test. We used SPSS 20.0 (SPSS Inc., USA) for all statistical analyzes and defined significance as a P-value of less than 0.05.

3. Results

3.1. Histopathological findings

Of the 200 cases, their mean age was 41 years (range: 20–80). proliferative endometrium, secretory endometrium, endometrial hyperplasia with atypia, low grade endometrial carcinoma and high grade endometrial carcinoma were diagnosed in 21%, 31.5%, 22.5%, 12.5% and 12.5% respectively. Table 1 shows the histopathological findings of the 200 patients included in this study.

3.2. Correlations between VDR and CRPB1 expressions and the histopathological diagnosis of the specimens (Fig. 1)

From 200 cases of the immunohistochemistry staining, VDR hasn't expressed in 35 (35/42, 83.3%) cases with proliferative endometrium and 13 (13/63, 20.6%) cases with secretory endometrium, while immunoreactivity was noted more in endometrial hyperplasia with atypia and endometrial carcinoma as VDR was strongly expressed in 8 (8/45, 17.8%) cases with endometrial hyperplasia, 15 (15/ 25, 60%) cases with low-grade endometrial carcinoma and 22 (22/25, 88%) cases with high-grade endometrial carcinoma (Table 2). The difference in

Table 1
The histopathological findings of the 200 patients included in this study.

Variable	No of cases	Percentage
Age (years)		
Mean	41	
Range)median)	20–80(43)	
Proliferative endometrium	42	21%
Secretory endometrium	63	31.5%
Endometrial hyperplasia	45	22.5%
Low grade endometrial carcinoma	25	12.5%
High-grade endometrial carcinoma	25	12.5%

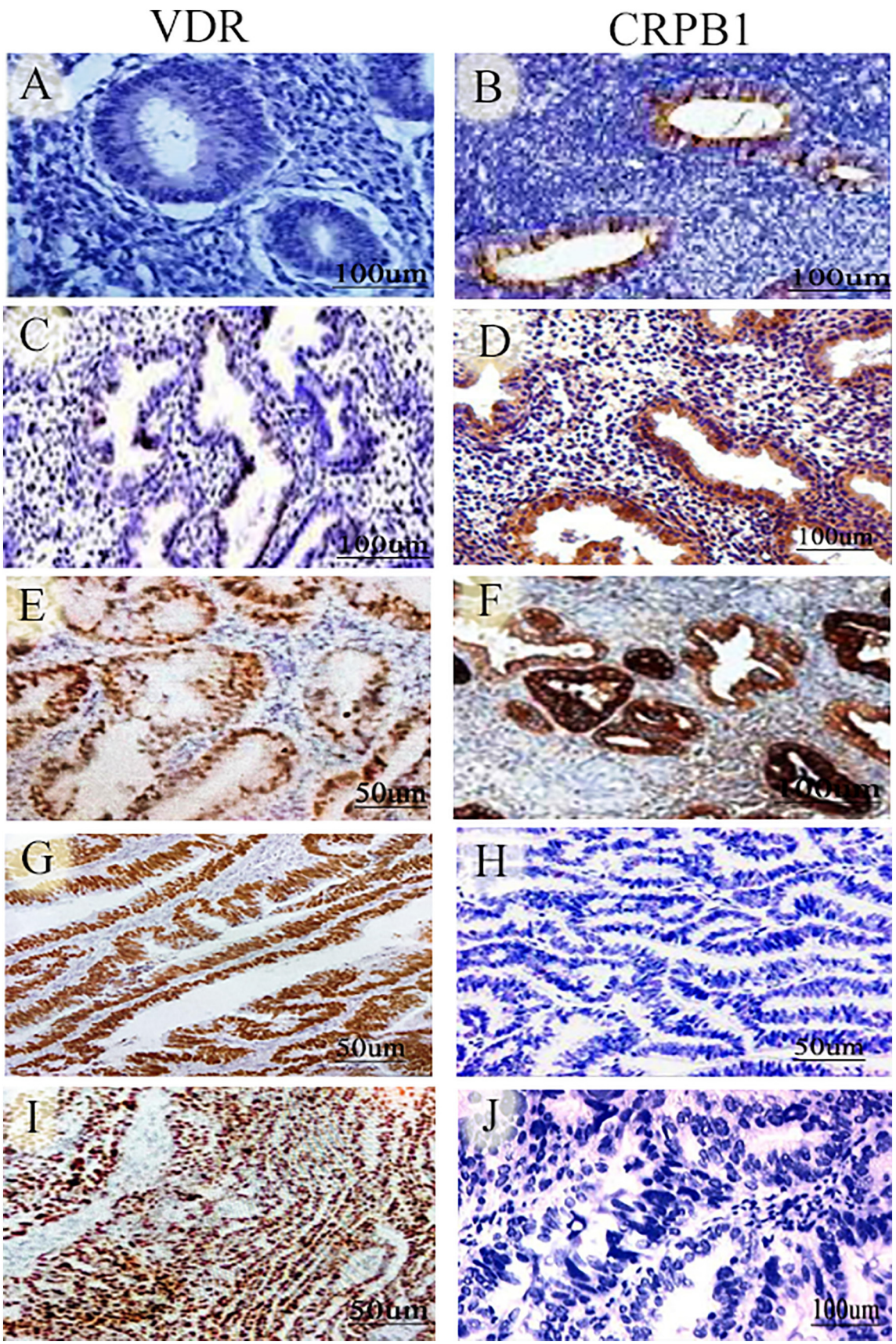

Fig. 1. Endometrium sections immunostained with VDR and CRBP-1 molecular markers:
(A and C) shows a negative expression of VDR in proliferative and secretory endometrium respectively (400×), (E) shows a moderate expression of VDR in endometrial hyperplasia (200×), (G and I) shows strong expression of VDR in low-grade endometrial carcinoma and high-grade endometrial carcinoma respectively (400×), (B, D, and F) shows strong expression of CRBP-1 in proliferative, secretory endometrium and endometrial hyperplasia respectively (400×), (H and J) shows a negative expression of CRBP-1 in low-grade endometrial carcinoma and high-grade endometrial carcinoma respectively(400×).

Table 2
Correlations between VDR and CRPB1 expressions and the histopathological diagnosis of the specimens.

Histopathological diagnosis	N	The overall score of protein expression									
		VDR					CRPB1				
		Negative	Positive			P-value [a]	Negative	Positive			P-value [b]
			+	++	+++			+	+	+++	
Proliferative endometrium	42	35(83.3)	7(16.7)	0(0)	0(0)		0(0)	0(0)	5(11.9)	37(88.1)	
Secretory endometrium	63	13(20.6)	50(79.4)	0(0)	0(0)		0(0)	0(0)	7(11.1)	56(88.9)	
Endometrial hyperplasia	45	0(0)	4(8.9)	33(73.3)	8(17.8)	< 0.001 *	0(0)	13(28.9)	29(64.4)	3(6.7)	< 0.001 *
Low-grade endometrial carcinoma	25	0(0)	0(0)	10(40)	15(60)		2(8)	23(92)	0(0)	0(0)	
High-grade endometrial carcinoma	25	0(0)	0(0)	3(12)	22(88)		20(80)	5(20)	0(0)	0(0)	

* Significant, Using Percentage of Row, + = mild positivity, ++ = moderate positivity, +++ = strong positivity.
[1] Chi-square test.
[2] Fisher's exact test.

immunoreactivity between the groups was significant (P < 0.001). While CRPB1 overexpression (strong expression) was noted in cases with proliferative endometrium, secretory endometrium and endometrial hyperplasia with atypia 37 (37/ 42, 88.1%), 56 (56/63, 88.9%) and 3 (3/45, 6.7%) cases respectively and all case with low-grade endometrial carcinoma and high-grade endometrial carcinoma showed negative expression (Table 2). The difference in immunoreactivity between the groups was significant (P < 0.001).

3.3. Correlations between VDR and CRPB1 expressions

We observed a high percentage of positive VDR expression in endometrial tissue samples with negative and mildly positive expression of CRPB1 and vice versa. The difference in immunoreactivity between VDR and CRPB1was significant (P < 0.001) (Table 3).

4. Discussion

Endometrial carcinoma occupies the fifth most common cancer of women worldwide [19,20]. The five-year survival rate ranges from 74% to 91% in patients who diagnosed in the early stages of endometrial carcinoma [19]. There are many factors responsible for developing endometrial cancer are older age, nulliparity, estrogen-only hormone replacement therapy, diabetes, and obesity [21].

In the present study, we analyzed the expression of VDR and CRBP-1 in endometrial carcinomas in comparison with that of normal (proliferative and secretory) and hyperplastic endometrium.

Ecological studies confirmed that UV exposure affects the risk of developing endometrial cancers by increasing levels of vitamin D. Women who are living in higher latitudes have a higher risk of developing endometrial cancers than those living in lower latitudes [22]. An inverse association between endometrial cancer incidence and UVB irradiance was demonstrated in a study done by [22].

Our results showed that VDR negative immunoreactivity was detected in the majority of normal endometrial Cells, while VDR expression increased in endometrial hyperplasia)17.8% of cases show strong expression) and this feature progress by endometrial carcinoma progression as 88% of cases with high-grade endometrial carcinomas have a strong expression in comparison to 60% of cases with low-grade endometrial carcinoma. These results were in agreement with a study done by [23] who published that VDR levels in endometrial cancer are significantly higher than in the control endometrium. Studies examining the role of VDR in endometrial cancer are sparse, while most studies done on VD proposed that VD has anti-proliferative effect in endometrial cancer cell lines, mainly a mechanism of growth arrest [24] or apoptosis [25] as calcitriol treatment-induced arrest of the cell cycle in endometrial cancer cells by suppressing some regulators of progression at the cell cycle. Inversely, A Swedish study suggested that endometrial cancer risk is decreased by 40% by using sunbeds more than three times per year [26]. While in 2010, a study was done on 830 endometrial cancer cases measuring circulating concentrations of 25(OH)D supported that vitamin D did not have any protective role against endometrial cancer [27].

Table 3
Correlations between VDR and CRPB1 expressions.

VDR expression	CRPB1 expression				
	Negative	Weak	Moderate	Strong	P
Negative	0(0)	0(0)	3(13.6)	19(86.4)	
Mild	0(0)	1(2.4%)	20(48.8)	20(48.8)	< 0.001 *
Moderate	6(14.6)	9(22)	21(51.2)	5(12.2)	
Strong	42(43.8)	51(53.1)	2(2.1)	1(1)	

* Significant (Chi-square test), P=P value.

As vitamin A metabolism is complex, the role of CRBP-1 in retinoid signaling remains controversial, although numerous studies were done over the last three decades on its binding properties [28]. CRBP-1 gene function in controlling the cell bioavailability of vitamin A suggests that it has a role in the inhibition of early steps of cancer transformation as downregulation of CRBP-1 expression was detected in a series of tumors: breast, endometrial, ovarian, prostate, astrocytic gliomas, renal cancer, larynx cancer, nasopharyngeal carcinoma, cervical cancer, gastrointestinal carcinomas and lymphoma [13]. Many studies highlighted the role of CRBP-1 signaling in the progression of cancer during the last years, but the mechanisms affect carcinogenesis are not being fully elucidated.

In the present study, CRBP-1 negative immunoreactivity was detected in the majority of endometrial carcinomas with a progressive decrease of observed in less differentiated endometrial tumors (80% of cases with high-grade endometrial carcinomas and 8% of cases with low-grade endometrial carcinomas) but none in endometrial hyperplasia with atypia and normal endometrium. This striking overall difference in CRBP-1 expression in low grade and high-grade endometrial carcinomas reflects the differences in their risk factors and molecular pathogenesis as the absence of CRBP-1 expression in most high-grade carcinomas further supports the presence of distinct molecular carcinogenic pathways. Our results are in agreement with previous reports documenting a loss of CRBP-1 expression in breast and ovarian carcinoma [28,29]. Also [30] suggested that the absence of CRBP-1 expression in less differentiated carcinomas resulting in a sort of intracellular hypovitaminosis, since normal or even excess of levels of retinol cannot promote epithelial differentiation. In this point of view, screening for CRBP-1 expression may represent a potential target of therapeutic strategies at influencing the growth of endometrial cancer cells through an increase of retinoic acid bioavailability and thus arrest the progression of endometrial carcinomas.

Increased VDR expression and reduced CRBP-1 expression are

associated with malignant features of the endometrium with a significant statistical difference of immunoreactivity between groups of normal endometrium, hyperplastic changes & carcinoma. Our data suggested that increased VDR expression is partly associated with endometrial cancers through a premalignant phase. Also, increased VDR and reduced CRBP-1 expression are associated with the progression of endometrial carcinoma to higher grades. Furthermore, VDR and CRBP-1 immunodetection can be considered as an additional useful tool for endometrial carcinoma grading and may help in the detection of areas of differentiation, which could not be easily identified by routine histopathological. Further studies are needed to define the biological role of VDR and CRBP-1 expression with different patterns and possible implications in a pharmacological strategy aiming to counteract endometrial carcinomas progression.

Author contribution

- Dalia M. Badary and Hisham Abou-Taleb have contributed significantly in all steps including conception and study design, acquisition and analysis of data; drafting the manuscript, tables, and figures.
- Hisham Abou-Taleb has contributed significantly to conception and study design and final revision.
- Dalia M. Badary has contributed to data collection, drafting the manuscript, tables, and figures.
- All authors agree with the content of the manuscript.

Ethics approval and consent to participate

This research received ethics approval from the Committee of Medical Ethics of Faculty of Medicine, Assiut University.

Declaration of competing interest

The authors declare that they have no competing interests.

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