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ARTICLE

Clinicopathologic Evaluation of CDw75 Antigen Expression in Colorectal Adenocarcinomas*

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CDw75, a B lymphocyte surface antigen, is a sialylated carbohydrate epitope, which is generated by the enzyme β galactosyl α2,6 sialyltransferase (Sia-T1). In colon carcinomas, although higher levels of Sia-T1 has been described and found to be correlated with metastatic potential of tumor cells, the expression of CDw75 antigen still remains unknown. To address this issue, we investigated immunohistochemically CDw75 antigen expression in 195 colorectal adenocarcinomas and their nodal metastases. The correlation between CDw75 antigen expression with selected clinicopathologic variables was analyzed by using Chi-square and Fisher's exact tests. Positive staining was observed in 101 cases. Non-neoplastic mucosa was negative consistently. The frequency of positivity was decreased according to the degree of differentiation ($p<0.001$). Antigen expression was found to be associated with deeper penetration ($p<0.006$), positive lymph nodes ($p<0.001$), distant metastases ($p<0.006$) and advanced stage ($p<0.001$). Same relationships were detected in well and moderately differentiated tumors when CDw75 immunoreactivity was evaluated in each histologic grade separately. Our findings indicate that CDw75 antigen expression may be a good indicator of the biological aggressiveness of colorectal adenocarcinomas especially in tumors with well and moderately differentiated morphology. (Pathology Oncology Research Vol 8, No 3, 175–182)

Keywords: CDw75 antigen, β-galactosyl α2,6 – sialyltransferase, colon carcinoma, sialyltransferases, immunohistochemistry

Introduction

Compositional and structural alterations in the oligosaccharide proportions of cell surface glycoproteins are frequently observed during neoplastic transformation and metastasis formation.[11,15,19,20,44] These alterations result either de-novo formation of glycoproteins that not exist in the parenteral cell or re-expression of glycoconjugates characteristic of fetal life.[15,20,40,44,49] In malignant tumors a well-documented change of this kind is the production of extensively sialylated sugar chains in cell surface glycoconjugates through the action of sialyltransferases, members of glycosyltransferase family of enzymes. [7,14,34,38,42,46]

Recently, abnormal levels of sialyltransferases were described in human colon cancer. β-galactosyl α2,6-sialyltransferase (Sia-T1) that mediates the transfer of sialic acid onto Gal βi4GlcNAc termini of N-linked oligosaccharides is amongst them.[4,18,28] Sia–T1 plays an important role in the generation of α2,6 sialylated sugar chains in the cell surface glycoproteins.[39,47] In previous works, Sia-T1 activity was found to be higher in human colorectal cancer than adjacent colonic mucosa.[4,31] It was considered as an onco-developmentaly regulated enzyme.[4-6] Moreover, in colon carcinoma cell cultures, the relationship between increased Sia-T1 activity and metastatic abilities of malignant cells is noted. [21,28,29] In a few study, an increased α2,6 sialylated sugar residues generated by this enzyme has also been described on histochemical and ultrastructural basis in colon carcinoma.[7,42]

CDw75 is a human leukocyte surface antigen which present in surface immunglobulin-positive B lympho-

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Abbreviations

Sia-T1: β galactosyl α2,6 sialyltransferase

cytes, is a sialylated glycoconjugate requiring the enzymatic activity of Sia-T1 for its elaboration.[1,35] Four monoclonal antibodies identical in reactivity and recognizing a single epitope (HH2,LN-1,EBU-141 and OKB-4), are used to define this antigen immunohistochemically.[1,35]

The presence of CDw75 in solid organ tumors was evaluated in some studies.[9,12,32,41] In gastrointestinal system, the presence of this antigen was investigated in gastric carcinoma and CDw75 expression was found to be a hallmark of neoplastic transformation and closely correlated with aggressive behavior of tumor cells and poor prognosis.[9,12]

Although the differences in the expression of sialylated glycoconjugates and their correlation with metastatic properties of colorectal carcinomas were extensively studied, neither the presence of CDw75 antigen nor its correlation with clinicopathologic characteristics is delineated yet in this disease. Therefore this study was undertaken to investigate CDw75 antigen expression in

Table 1. **Correlation between CDw75 antigen expression and clinicopathologic parameters in colorectal adenocarcinomas**

	No[a]	*CDw75 negative*	*%*	*CDw75 positive*	*%*	*p value*
Gender						
Male	103	52	50.5	51	49.5	ns[b]
Female	92	42	45.7	50	54.3	
Age						
<49[c]	98	49	50	49	50	ns
49	97	45	46.4	52	53.6	
Tumor location						
Distal	99	43	43.4	56	56.6	ns
Proximal	96	51	53.1	45	46.9	
Tumor Diameter						
<5.2cm[c]	93	46	49.5	47	50.5	ns
5.2cm	102	48	47.1	54	52.9	
Histologic subtype						
Well	61	9	14.8	52	85.2	
Moderate	42	13	31	29	69	
Poor	38	25	65.8	13	34.2	0,001
Mucinous adenocarcinoma	30	27	90	3	10	
Signet ring cell adenocarcinoma	24	20	83.3	4	16.7	
Depth of wall invasion						
T1	35	26	74.3	9	25.7	
T2	42	19	45.2	23	54.8	
T3	57	26	45.6	31	54.4	0.006
T4	61	23	37.7	38	62.3	
Lymph node metastasis						
Absent	95	63	66.3	32	33.7	0,001
Present	100	31	31	69	69	
Distant metastasis						
Absent	133	71	53.4	62	46.6	0,006
Present	62	23	37.1	39	62.9	
Lymphatic invasion						
Absent	134	73	54.5	61	45.5	0,001
Present	61	21	34.4	40	65.6	
Vascular invasion						
Absent	122	57	46.7	65	53.3	ns
Present	73	37	50.7	36	49.3	
Stage						
SI	38	28	73.3	10	26.3	
SII	35	20	57.1	15	42.9	0,001
SIII	60	23	38.3	37	61.7	
SIV	62	23	37.1	39	62.9	

[a] Number of cases, [b] Not significant, [c] Mean ± Standard deviation

colorectal adenocarcinomas. The relationship between the presence of this antigen and clinicopathologic characteristics was also evaluated.

Materials and Methods

One hundred and ninety five cases with colorectal adenocarcinoma, diagnosed and treated between 1982 and 2001 at the Departments of Pathology and General Surgery, Akdeniz University Hospital, Antalya, Turkey were included in this study. The patients had undergone curative or palliative surgical resection combined with lymph node dissection. The mean age was 48.5 years (standard deviation: 12.25 years, range, 22-81 years) at the time of operation and with a male- to-female ratio of 1.11.

Five micrometer thick HE stained sections from all tissues originally fixed in buffered formalin and embedded in paraffin were selected. Histolological classification and staging were performed according to WHO classification and TNM system, respectively.[2,26] Tumors located in caecum, ascending colon and transverse colon referred as ''proximal'' and tumors located in descending colon, sigmoid colon and rectum referred as ''distal''. The clinicopathologic characteristics of our cases are summarized in *Table 1.*

Sections from selected paraffin embedded tissue blocks from each tumor, normal mucosa from surgical resection margins and involved lymph nodes, were deparaffinized and heated in a microwave oven for 10 minutes to retrieve antigens. Pronase treatment (Protease 24 0,1%, Sigma, Deisenhofen, Germany) was done for 15 minutes. Endogenous peroxidase activity was blocked using 3% hydrogen peroxide in methanol for 10 minutes at 20°C. Each step of incubation was followed by thorough washing the slides in distillated water and phosphate buffered saline (PBS) (0,001%, Sigma). After incubation with primary antibody LN-1 (mouse monoclonal, dilution: 1/50, Zymed) for 30 minutes, sections were allowed to react with secondary biotinylating antibody for 15 minutes and streptavidin for 15 minutes. Finally, all slides were treated with DAB reagent to develop color and counterstained with hematoxylin.

Negative controls were performed by using non-reactive IgM of the same concentration as the primary antibody. In all series relevant positive controls included sections from lymph nodes.

The staining pattern was classified semiquantitatively as follows: (–): tissue specimens without staining, (+): tissue specimens with less than 10% of the cancer tissue stained, (++): tissue specimens with 10 to 50% of the cancer tissue stained, (+++): tissue specimens with more than 50% of the cancer tissue stained.

Chi-square test and Fisher's exact test were performed to evaluate the association of CDw75 antigen expression with clinicopathological parameters.

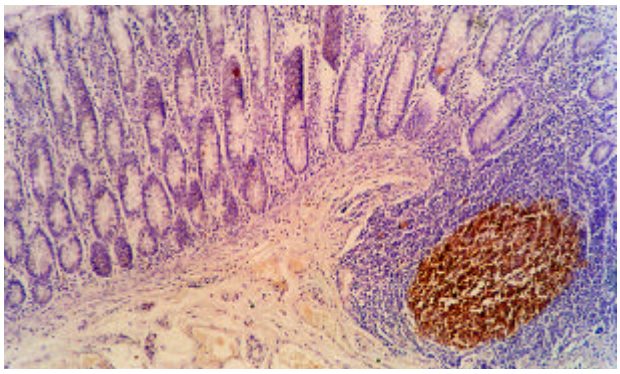

Figure 1. Section from normal colonic mucosa. Lymphocytes in a lymphoid follicle show positive staining for CDw75 antigen. However, there is no immunoreactivity in non-neoplastic mucosa (x 100).

Results

In specimens from normal mucosa CDw75 expression was not detected in any case. Only germinal center cells of lymphoid follicles located in submucosa were positive consistently (*Figure 1*). In contrast CDw75 immunoreactivity was detected in 101 cases of carcinomas. Tumor cells with CDw75 expression were heterogeneously distributed in tumor tissues and did not preferentially located in any level of the colonic wall. In all positive cases, immunoreaction was either located on cytoplasm or plasma membrane of the cells (*Figure 2*).

Degree of staining was as follows: –: 47.2%; +: 15.3%; ++: 18.5%; +++: 19%. Expression intensity was weak in 23, moderate in 44 and strong in 34 cases. Any relationship was not found between degree or intensity of staining and the clinicopathologic factors.

As shown in Table I; there was no significant correlation between the presence of CDw75 expression in neoplastic tissue and clinicopathologic factors such as, age, gender, localization, tumor size, and vascular invasion.

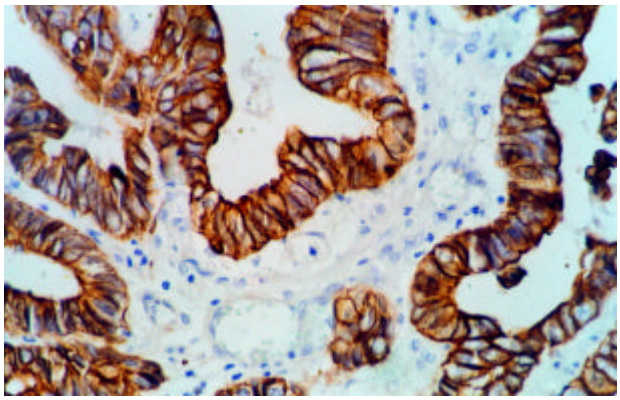

Figure 2. Well-differentiated adenocarcinoma with strong cytoplasmic and membrane staining for CDw75 antigen (x 400).

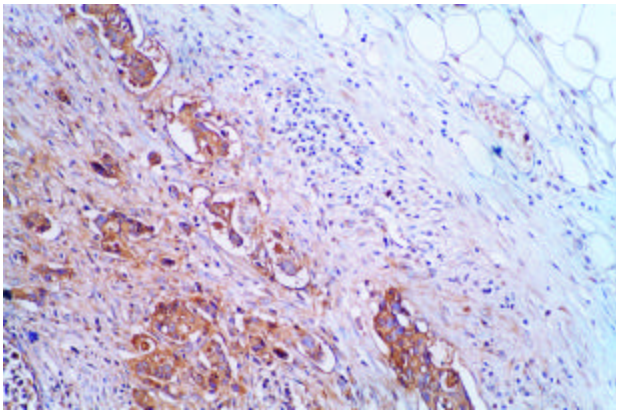

Figure 3. Strong CDw75 positivity on tumour cell invading serosa and lymphatics in a case of moderately differentiated adenocarcinoma (x 200).

CDw75 staining was detected in 10% of mucinous adenocarcinomas and 16.7% of signet ring cell carcinomas, respectively. However, in ordinary adenocarcinomas, CDw75 positivity was detected in 94 of 141 (66.7%) of cases and antigen expression was found to be inversely correlated with degree of differentiation ($p<0.001$) *(Table 1).*

CDw75 expression was more frequent in tumors with deeper penetration ($p<0.05$). Statistically significant correlations were also detected between CDw75 expression and lymph node metastasis, lymphatic invasion, the presence of distant metastasis and advanced stage ($p<0.05$) *(Table 1).*

In cases with nodal involvement, when the presence of CDw75 expression in metastatic and primary tumor cells were compared, in all CDw75 positive tumors (69 cases), antigen expression was also detected in their nodal metastasis *(Figure 3).* In contrast, any positivity was not detected in metastatic tumor cells in whole tumors without CDw75 immunoreactivity (31 cases).

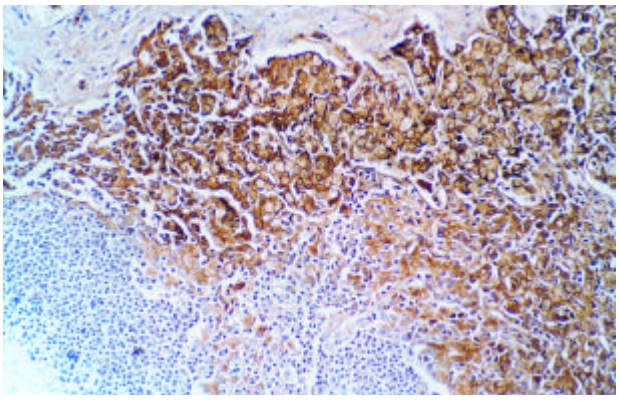

Figure 4. Nodal involvement in a case of signet ring cell type adenocarcinoma. Metastatic tumor cells show heterogeneous cytoplasmic and membrane positivity for CDw75 antigen. In this case primary tumor cells were also positive (x 250).

We also expected to determine, a correlation, if any, between antigen expression and clinicopathologic parameters in tumors with different morphologic grade. For this reason cases were divided in three groups according to the degree of differentiation of their tumors. Statistical analysis was performed in each of these groups separately. As a small number cases were found to be stained with CDw75 antigen in mucinous adenocarcinomas (3 cases) and signet ring cell carcinomas (4 cases), further statistical analysis was not performed in these groups.

Statistical analysis revealed that in well-differentiated adenocarcinomas antigen expression was related to dept of wall invasion, lymph node metastasis, lymphatic invasion, the presence of distant metastasis and advanced stage ($p<0.05$) *(Table 2).* The same correlations were also detected in moderately differentiated tumors ($p<0.05$) *(Table 2).* However any relationship was not detected between CDw75 antigen expression and clinicopathologic parameters in poorly differentiated adenocarcinomas *(Table 2).*

Discussion

In this study, CDw75 positivity was detected in 51.8% of carcinomas. In contrast, epithelial cells of the normal colon lacked positivity with this antigen. The expression of many antigens by normal colonic epithelium and carcinomas has been extensively studied by numerous investigators. It has been demonstrated that some of these antigens are absent in normal mucosa but expressed in fetal colon, colorectal adenomas and carcinomas, and are considered oncofetal tumor associated antigens.[3,22-24,27,30] The present study, partly confirm these observations with CDw75 antigen. Although in our study CDw75 expression in fetal colon and adenomas were not evaluated and additional research is warranted, we interpret the complete absence of this antigen in normal mucosa to be supportive of CDw75 expression as a marker of malignant transformation.

CDw75 is a sialoglycoconjugate which requires the enzymatic activity of Sia-T1 for its elaboration.[1,35] An enhanced activity of Sia-T1, is represented by the appearance of CDw75 antigen on the cell surface[4,13]. Recent biochemical studies demonstrated that Sia-T1 is an oncodevelopmentally regulated enzyme.[4-6] The activity of Sia-T1 is higher in fetal colonic mucosa, but progressively declines between suckling and weaning transition in rats and the enzyme activity is low but biochemically detectable in normal colon.[5] Furthermore, human colon cancer tissue express a Sia-T 1 activity three to six times higher than that of normal tissues and consequently an increased degree of α2,6 sialylated glycoconjugates.[4,31,36] From this point of view, in our series higher frequency of CDw75 antigen expression in carcinoma and lack of pos-

itivity in normal mucosa may be a reflection of the increased Sia-T1 activity in colorectal adenocarcinomas.

In this study, CDw75 antigen positivity was detected in a few cases of mucinous adenocarcinomas and signet ring cell carcinomas. However, in our series 94 of 101 CDw75 positive tumors were ordinary adenocarcinomas. Moreover, CDw75 expression in these cases was more frequent in well-differentiated tumors and the frequency of staining was decreased according the degree of differentiation ($p<0.001$). Although in colon carcinoma a correlation between differentiation of tumor cells and CDw75 antigen expression was not described, in previous studies, a relation between Sia-T1 activity and degree of cell differentiation has been demonstrated. Dall'Olio et al[8] observed that in human colon carcinoma CaCo-2 cells, the cell associated and medium release of Sia-T1 increases with the degree of cell differentiation and differentiated cells contain a higher number of enzyme molecule than those undifferentiated cells. Similarly, Gangopadhyay et al[17] by using a murine monoclonal antibody, 6B9 of IgM isotype against Sia-T1, was also detected the differentiation dependent expression of this enzyme. Parallel to these observations, our findings demonstrate that CDw75 expression in colon carcinoma is not associated with extreme dedifferentiation of tumor cells and support an increased Sia-T1 activity in tumors with well differentiated and moderately differentiated morphology.

Table 2. **Correlations between CDw75 expression and clinicopathologic parameters in colorectal adenocarcinomas with different morphologic grades**

	Well differentiated adenocarcinomas (n: 61)			*Moderately differentiated adenocarcinomas (n: 42)*			*Poorly differentiated adenocarcinomas (n: 38)*		
	Negative	*Positive*	*p value*	*Negative*	*Positive*	*p value*	*Negative*	*Positive*	*p value*
Gender									
Male	4	26	ns[a]	6	16	ns	14	5	ns
Female	5	26		7	13		11	8	
Age,years									
< median value[b]	5	24	ns	3	12	ns	15	7	ns
= median value	4	28		10	17		10	6	
Tumor location									
Distal	5	36	ns	4	11	ns	11	7	ns
Proximal	4	16		9	18		14	6	
Tumor diameter									
< median value[c]	4	22	ns	7	18	ns	15	6	ns
= median value	5	30		6	11		10	7	
Depth of wall invasion									
T1+T2	9	18	0.001	10	9	0.006	9	6	ns
T3+T4	–	34		3	20		16	7	
Lymph node metastasis									
Absent	8	17	0.002	13	5	0.001	12	6	ns
Present	1	35		–	24		13	7	
Distant metastasis									
Absent	9	33	0.029	13	18	0.01	19	10	ns
Present	–	19		–	11		6	3	
Lymphatic invasion									
Absent	9	31	0.019	13	16	0.004	18	10	ns
Present	–	21		–	13		7	3	
Vascular invasion									
Absent	5	32	ns	10	18	ns	21	10	ns
Present	4	20		3	11		4	3	
Stage									
SI + SII	8	13	0.001	13	4	0.001	9	6	ns
SIII + SIV	1	39		–	25		16	7	

[a] Not significant, [b] 54.3, 49.8 and 51.7 years in well, moderately and poorly differentiated adenocarcinomas, respectively
[c] 4.3 cm, 5.4 cm and 5.1 cm in well, moderately and poorly differentiated adenocarcinomas, respectively.

Quantitative differences in cell surface sialic acid and qualitative changes in sialylated glycoconjugates are commonly associated with aggressive behavior and metastatic potential in tumor cells.[16,21,28,29,50] Recent studies on colon carcinomas have been demonstrated a close correlation between increased expression of various sialylated glycoconjugates such as, sialyl Lewis x (Le^x), Lewis a (CA-19-9), sialylated Tn antigen and metastatic behavior of tumor cells.[25,37,48] Experimental studies also revealed that, colon cancer cells with a high expression of α2,6 sialylated glycans are more tumorogenic and metastatic in mice.[21,29] Besides, accumulated evidences indicate that in colon cancer cells a shift to enhanced α2,6-sialylation of glycoproteins via N-glycan specific α2,6-sialyltransferase results with an increase in the amount of the cell surface sialic acid which promotes invasive properties of tumor cells and consequently metastasis.[21,28,29] In our series CDw75 expression was closely correlated with lymphatic invasion, lymph node and distant metastasis. Moreover, same relationships were detected when CDw75 expression was evaluated in well-differentiated tumors and moderately differentiated tumors separately. However any correlation was not detected between CDw75 expression and clinicopathologic parameters in poorly differentiated tumors. The results of the previous studies and the present one suggest that CDw75 expression on well differentiated and moderately differentiated colon carcinoma cells may reflect an increased tendency to metastasize via lymphatics, possibly depending sialic acid moiety of the antigen.

In our cases we observed CDw75 antigen expression more frequently in tumors with deeper penetration. In an elegant study Ming et al[33] showed that in normal colonic mucosa Sia-T1 is downregulated by deoxycholate (DOC), a bile acid and TPA (12-O-tetradecanoylphorbol-13-acetate) a phorbol ester which are present in fecal constituents. They hypothesized that, as colorectal tumor grows their progressive removal from fecal milieu that normally down regulates Sia-T1 may favor invasion and metastasis. This finding lead us to speculate that in colon carcinoma, enhanced CDw75 expression in tumors with deeper penetration could be a reflection of the increase Sia-T1 activity and invasive properties of tumor cells, especially in tumors with well differentiated and moderately differentiated morphology.

Recent studies indicated that sialylation of oligosaccharide proportion of tumor cells play an important role in the expression of metastatic phenotype and primary tumor cells with certain glycoconjugate expression had ability to metastasize.[10,50] In our series 31 of 100 cases with nodal involvement, CDw75 expression was not detected in primary and metastatic tumor cells. In contrast in 69 tumors with CDw75 expression, antigen expression was also detected in their respective metastasis. As metastatic process is a complex event and is not completely clarified, it is not possible to conclude with the current findings that CDw75 antigen is a unique molecular phenotype that determines colorectal cancer metastasis. Recently, CD22 a B lymphocyte surface receptor described as a sialic acid binding lectin.[43,45] Stamenkovic et al[45] demonstrated that CD22 is a specific receptor for α2,6 sialylated glycoconjugate, which interacts with CDw75 on B, cells and suggested a possible a role for CD22-CDw75 molecules in B cell interactions. At present, is not known whether the presence of CD22 antigen in cells of the primary target organs for metastasizing colon carcinoma and CDw75 expression in metastatic tumor cells have a role in tumor progression via CD22-CDw75 interactions, or not. However our findings support that CDw75 antigen maintains a stable genotypic/phenotypic pattern during colorectal adenocarcinomas progression, as described previously on gastric malignancies.[9,12]

Studies on gastric carcinoma demonstrated that besides its association with the metastatic properties of tumors cells, CDw75 antigen expression is a good indicator of prognosis in patients with gastric carcinoma.[9,12] Unfortunately in the present study in a great majority of cases no survival data was available. We propose that in colorectal adenocarcinoma further studies are needed to establish the relationship between CDw75 antigen expression and prognosis particularly in well differentiated and moderately differentiated tumors.

In colon cancer, the presence of many tumor associated antigens and their relationship with clinicopathologic parameters have been described. In this context, we evaluated the expression of CDw75 antigen in patients with colorectal adenocarcinomas. Although further studies including fetal colonic tissue, adenomas, and adenocarcinomas is necessary to establish the significance of CDw75 expression during neoplastic transformation in colonic mucosa, our findings demonstrate that antigen expression is closely related to malignancy. The significant association of CDw75 positivity in primary tumor cells and in their respective metastasis suggest that antigen expression maintains a stable genotypic/phenotypic pattern during metastasis formation. Our results also indicate that, in colorectal adenocarcinomas immunohistochemical evaluation of CDw75 expression might be valuable to determine aggressive behavior of malignant cells in tumors with well and moderately differentiated morphology.

Further investigations in larger series with longer follow-up might provide not only better understanding the role of CDw75 expression in neoplastic transformation, but also might help to determine the predictive value of the antigen expression in prognosis of patients with colorectal adenocarcinomas, especially in tumors with low and moderately differentiated morphology.

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