



Expression of FasL in Proliferation of Retinoblastoma Cells: A Mechanism Fas Counterattack

Retinoblastoma Hücrelerinin Çoğalmasında FasL Ekspresyonu: Fas Atağının Mekanizması

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Cukurova Medical Journal 2014;39(4):788-794.

ABSTRACT

Purpose: The aim of this study were to determine the association of increasing of FasL with increasing proliferation of retinoblastoma cells.

Materials and Methods: The protein expression was analyzed in 30 retinoblastoma samples from paraffin block using immunohistochemical method for evaluation of FasL, CDK4, and Ki-67 expression.

Results: Among 30 retinoblastoma samples, FasL expression majority was negative in 33.3 % (10 samples) and strong in 36,8 % (11 samples). CDK4 majority 53,3% was weak expression and Ki-67 was high expression also in 53,3% (16 samples). The expression of FasL was significantly related to CDK4 (r: 0.363; p: 0.048). The CDK4 was also significantly related to Ki-67 expression (r: 0.601; p: 0.000).

Conclusion: The increasing of FasL on the mechanism Fas counterattack induces proliferation of retinoblastoma cells.

Key Words: FasL, proliferation, counterattack, retinoblastoma.

ÖZET

Amaç: Bu çalışmanın amacı artan FasL ekspresyonu ile retinoblastom hücrelerindeki proliferasyon artışı arasındaki ilişkiyi incelemektir.

Materyal ve Metod: Ki-67, CDK4 ve FasL ekspresyon değerlerini incelemek için parafin blok yapılmış 30 retinoblastom örneğinin protein ekspresyon değerleri immünohistokimyasal analiz ile belirlendi.

Bulgular: 30 retinoblastom örneği arasından %33.3 ünde (10 örnek) FasL ekspresyonu yok iken %36,8 inde güçlü bir ekspresyon vardır. %53.3 (16 örnek) örnekte CDK4 ekspresyonunun zayıf olduğu görülürken Ki-67 ekspresyonunun yüksek olduğu tespit edilmiştir. FasL ekspresyonu yüksek oranda CDK4 ekspresyonu ile ilgilidir (r:0,363, p:0,048). Aynı zamanda CDK4 ün ekspresyonu da Ki-67 ekspresyonuyla ilgilidir (r:0,601, p: 0,000).

Sonuç: FasL atağı mekanizmasındaki artan FasL ekspresyonu retinoblastoma hücrelerinin proliferasyonunu indüklemektedir.

Anahtar kelimeler: FasL, proliferasyon, atak, retinoblastom

INTRODUCTION

Retinoblastoma has improved a survival rate of patients over years and been increasing in the developed countries because of no early method detection and proper therapy was. Retinoblastoma derives from retinal cell, expands to the posterior chamber of the eye, and invades through the sclera and along the optic nerve¹. Whenever tumor extends beyond the globe into the orbit, a combination of radiation therapy and chemotherapy has been used. Prognosis remains poor for patients with dissemination into the central nervous system (CNS) and those with distant metastatic disease². A protein expression in proliferation and apoptosis pathway of retinoblastoma must be known to diagnose retinoblastoma because the correct diagnosis determines the appropriate treatment and to find a drug of choice by chemotherapy^{3,4}.

Progressiveness and prognosis of cancer cells can be evaluated by the balance of proliferation and apoptosis cells⁵. An imbalance between apoptosis and proliferation of cancer cells will be aggressive. In malignant tumor retinoblastoma, the role of cytokines Ras, Raf, and MEK will stimulate Cyclin Dependent Kinase 4 (CDK4) and induce CDK2, then cause Rb phosphorylation and have no capacity to bind E2F to lead a proliferation cell⁶. Expression of Ki-67 was used for measurement of levels of proliferation in different kinds of cancer cells. Ki-67 was expressed in all phases of the cells, except G0 phase^{7,8}.

Fas ligand (Fas/CD95L/CD178) and its receptor, Fas (APO-1/CD95), are members of the tumor necrosis factor family. The FasL is a 40 kDa type I membrane glycoprotein and can be found in activated T lymphocytes and NK cells^{9,10}. Association of Fas and FasL activates its death domain and thus triggers a cascade of caspases that results in apoptosis cell. FasL is activator of extrinsic apoptosis in cancer cells¹¹⁻¹³. Recently

discovered that FasL expression on cancer cells was significantly different from the normal. There was a role immunologic system of Fas in cancer cells against T cells lymphocytes, especially of cytotoxic T cells^{10,13,14}. Many types of cancer have been shown to express FasL because activated lymphocytes express Fas. Increased of FasL by tumor cells may enable to kill the T cells and induce resistance and proliferation of tumor cells. But it is still unknown how the role of FasL against proliferation in retinoblastoma.

The aim of this study was to determine the association of increased of FasL with increased proliferation of retinoblastoma cells using immunohistochemical staining.

MATERIALS and METHODS

The study group of 30 retinoblastoma samples from patients paraffin block was carried out an exenteration and enucleation therapy in Dr. Soetomo General Hospital – Medical Faculty of Airlangga University, Surabaya, Indonesia. None had received chemotherapy and radiotherapy prior to tissue samples collection.

The protein expression was evaluated by immunohistochemical reaction using antibodies for FasL (polyclonal antibody Fas-L; P137 (BS1122) Bioworld Technology Inc. (1:50), CDK4 (polyclonal rabbit CDK4(c-22): sc-260 Santa Cruz Biotechnology, Inc. (1:100), and Ki-67(CRM325 AK,BK) Biocare(1:75). All samples were stained by using Labelled Streptavidin Biotin II (LSAB II) method. A colour reaction for peroxidase was developed with DAB as a chromogen. The sections were counterstained with Meyer's haematoxylin.

Immunohistochemical stainings were scored by semiquantitative methods per high power field of 400x. Fas and FasL positive immunological staining reactions presented brown granules in the cytoplasm and/or cell membrane. FasL expression were determined and assessed as negative (<5%), weak (6-25%), moderate (26-50%), and strong

(>50%)¹⁵. Nuclear accumulation of CDK4 was evaluated according to the criteria: negative (0%), weak (<25%), moderate (25-50%), and high (>50%)¹⁶. Ki-67 expression on nuclear cell as negative (0%), low (≤40%), and high (≥40%)¹⁷. Colon cancer tissue sections known to express FasL were used as positive control. Positive

control tissues included breast cancer for CDK4 expression and prostate cancer for Ki-67 expression. Statistical analysis was conducted by using Mann-Whitney U-test and Spearman's correlation test. A p-value of < 0,05 was considered statistically significant.

RESULTS

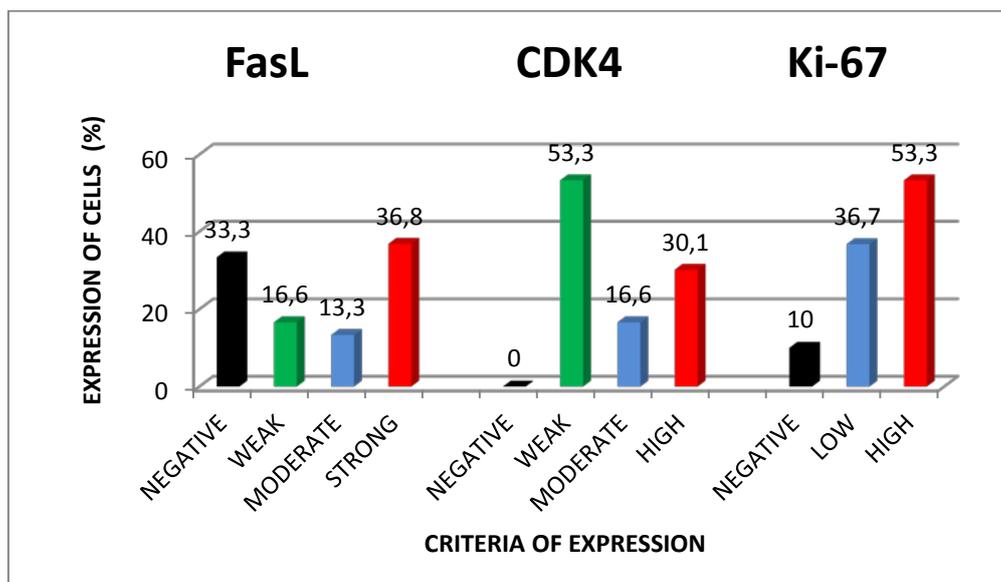


Figure 1. Expression of FasL, CDK4, and Ki-67 (n=30). Graphic box the percentage of cells on the criteria of expression.

There were 30 retinoblastoma samples to know protein expression of FasL, CDK4, and Ki-67 by immunohistochemical staining. Distribution of expression is presented in Figure 1. Among 30 retinoblastoma samples, FasL expression majority

was negative in 33.3 % (10 samples) and strong in 36,8 % (11 samples). CDK4 majority 53,3% was weak expression and Ki-67 was high expression in 53,3% (16 samples) as well.

Table 1. Correlations between FasL, CDK4, and Ki-67 in Retinoblastoma

	FasL	CDK4	Ki-67
FasL	-	p: 0.048*	p: 0.021*
CDK4	p: 0.048*	-	p: 0.000*
Ki-67	p: 0.021*	p: 0.000*	-

Note: (*) p<0,05 was considered statistically significant

FasL positive immunohistochemical staining was detected in retinoblastoma (Figure 2). A FasL positive immunohistochemical staining expressed a brown colour in the cytoplasm and cell

membrane. CDK4 and Ki-67 positive immunohistochemical staining expressed most tumor cells and a dark brown colour in nuclear cells (Figure 3 and Figure 4). Statistical analysis of

the results revealed statistically significant correlations of FasL, CDK4, and Ki-67. The expression of FasL was significantly related to

CDK4 (r: 0.363; p: 0.048). The CDK4 was also significantly related to Ki-67 expression (r: 0.601; p: 0.000).

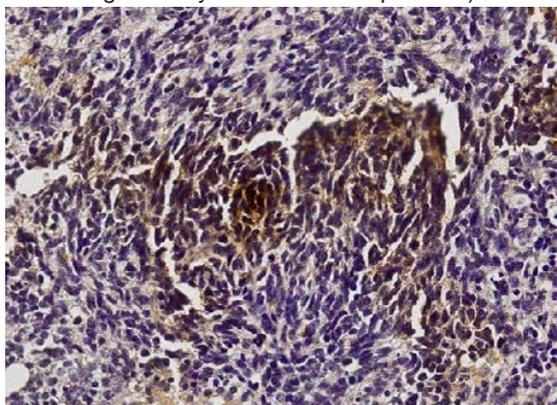


Figure 2. FasL staining in retinoblastoma expressed in cytoplasm and membrane cells. Original magnification x400.

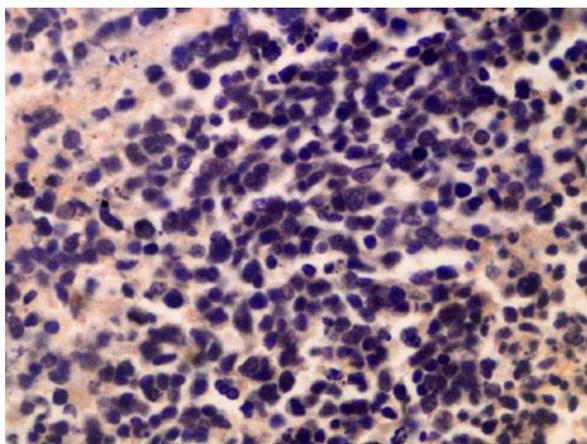


Figure 3. CDK4 staining was expressed many positive Cells with intact nuclei in retinoblastoma. Original magnification x400.

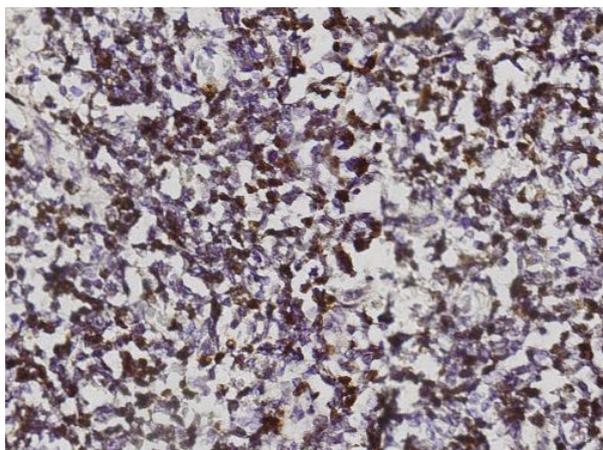


Figure 4. Ki-67 staining was expressed dark brown with intact nuclei in retinoblastoma. Original magnification x400.

DISCUSSION

On this study FasL expressed 66,7% of cells and 36,8% expressed strong. This indicated the role of FasL in immune mediated process of cancer cells¹⁸. Fas/FasL system plays an important role in the event of apoptosis in cytotoxic reaction involving the role of cellular immunologic against tumor cells^{10,14,19}. Associated of Fas and FasL activates its death domain and thus triggers a cascade of caspases that result in apoptosis cell. FasL is activator of extrinsic apoptosis in cancer cells. Apoptotic signal was sent by FasL-Fas associated to binding site of FADD protein and adapter enable Caspase-8 to Caspase-3 as apoptosis executor^{11,12}. Expression of FasL accordance with expression on cutaneous squamous carcinoma¹⁸, bladder cancer²⁰, cervical squamosa cancer²¹, and colon cancer²⁵.

Expression of CDK4 distribution showed that the majority of retinoblastoma is a weak and high expression. CDK4 is a key protein in G1 transition during cell-cycle progression. In complex with cyclin D, CDK4 phosphorylates G1-specific substrates, including the retinoblastoma protein (Rb). Rb phosphorylation in collaboration with cyclin D/CDK4 and cyclin E/CDK2 results in releasing of Rb from the E2F complex then the G1 phase of the cell enter S phase (DNA synthesis). If there was a mutation in the regulation of cyclin-D, then resulting in increased cell into S phase and the oncogenic transformation activities happen⁶.

Expression of Ki-67 showed that the majority of retinoblastoma is a high expression. The increasing Ki-67 proliferation marker indicate the aggressiveness of tumors that are characterized by an increase in the number of cells undergoing mitotic²⁰. Ki-67 was expressed on G1, S, G2, and mitotic phase but not on G0 phase^{7,8}. Ki-67 expression on retinoblastoma was accordance with expression on breast cancer⁷, colorectal carcinoma²³, and gastric carcinoma²⁴.

Our studies confirmed that expression of FasL was significantly related to proliferation of retinoblastoma. FasL significantly correlated with CDK4 and Ki-67. The system of associated Fas and FasL was responsible for cytotoxic T cell-mediated apoptosis and played a role in the immunologic homeostasis. Increasing expression of FasL was related to carcinoma that metastatic, such as found on stomach and esophagus cancer^{9,18,25}. Zhang et al., (2003) said that increased FasL expression on gastric cancer cells impact on immunologic response. A tumor cells having the immunity to kill a cytotoxic T cells. This mechanism called a Fas counterattack^{10,14}.

Normally, cytotoxic T cells produced FasL to bind a tumor Fas and induce apoptotic process of tumor cells. In Fas counterattack mechanism, tumor cells produce a MMP-7 to shedding a FasL cytotoxic T cells. Shedding of MMP-7 producing a soluble FasL which will inhibit an associated Fas/FasL then inhibit a FADD protein to bind receptors of death domain then the death signal of Caspase-8 to Caspase-3 interrupted^{10,27-29}.

Khriakumar et al., (2004) said that expression of FasL observed in many sites including the eye, inner ear, testis, or brain and also said that Fas/FasL pathway may be involved in the escape of human retinoblastoma cells from immune destruction. In eye, FasL is expressed on the surface of the parenchymal cells of the eye and FasL-positive cells may kill Fas-sensitive cells entering such sites. In early tumor development of retinoblastoma, proliferating Fas-positive retinoblastoma cells maybe recognized and eliminated by activated FasL expressing lymphocytes upon Fas ligation. Retinoblastoma cells expressing FasL may induce apoptosis of infiltrating Fas-positive T cells^{2,30-31}. Apoptosis in T cells increased with higher expression of FasL in cancer cells was accordance with expression on colorectal cancer^{25,32-33}.

In addition, some cancer cells have the ability to kill of cytotoxic T cells by producing FasL that binds Fas of cytotoxic T cells, then result T cell was apoptosis. The process of counterattack resulted in a increase a growth, invasion, and metastatic a tumor cell^{10,25,29}. Based on our data was concluded that increased of FasL on the mechanism Fas counterattack induces proliferation of retinoblastoma cells.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Geliş tarihi/Received on : 06.01.2014

Kabul tarihi/Accepted on: 14.02.2014