Short communication expression of glial fibrillary acidic protein in some tumors of dogs

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Abstract: Glial Fibrillary Acidic Protein (GFAP) is an intermediate filament protein in astrocytes of the Central Nervous System that has a molecular weight of approximately 50 kDa. GFAP, as an antigen in nervous tissue identification, is used as a reliable immunohistochemical marker for glial neoplastic cells and benign astrocytoma cells. In veterinary medicine breast cancers, skin neoplasms and lymphatic tumors are frequent. In scientific literatures it has been made clear that GFAP is a remarkable marker for the normal and neoplastic glial cells nevertheless there is no information about GFAP as a marker for the tumors not generally considered to be of glial origin. Therefore, in this study to determine GFAP in the several dog tumors except glial neoplasms was intended. The results of Sodium Dodecyl Sulfate Polyacrylamide Gel Electroforesis (SDS-PAGE) and Semi-Dry Blotting analysis using antibody for GFAP (immunoblotting) showed that GFAP is not being expressed by the 14 tumors which we studied. It has been observed that GFAP expression is in enough amount in the brain tissues. The fact that GFAP has not been identified in the tumor tissues we analyzed points out that this protein is specific to nervous system tissues.

Key Words: Glial fibrillary acidic protein, tumor, dogs, tumor markers

Bazı Köpek Tümörlerinde Glial Fibriler Asidik Protein Ekspresyonu

Öz: Glial Fibriler Asidik Protein (GFAP), molekül ağırlığı 50 kDa olan merkezi sinir sistemine ait astrositlerin intermediyer filament bir proteinidir. Sinir dokusunun tanımlanmasında bir antijen olarak kullanılan GFAP, merkezi sinir sisteminde glial orijinli neoplastik hücrelerin ve iyi huylu astrositom hücrelerinin lokalize edilmesinde immunhistokimyasal bir belirteç olarak kullanılır. Veteriner hekimliği alanında köpek tümörlerine özellikle deri, meme, kemik ve lenf dokularında sıklıkla rastlanır. Bilimsel literatürlerde GFAP'in glial orijinli normal ve neoplastik hücreler için güvenilir bir belirteç olduğu belirtilmektedir. Ancak merkezi sinir sisteminin dışındaki dokularda GFAP'in bir belirteç olabileceği hakkında herhangi bir bilgi yoktur. Bu nedenle bu çalışmada köpeklerden elde edilen ve çeşitli dokulardan orijin alan tümörlerde GFAP'in varlığı ve ayırıcı teşhisteki değerinin saptanması amaçlanmıştır. Elde edilen 14 adet tümörlü ve sağlıklı dokunun Sodyum Dodesil Sülfat Poliakrilamid Jel Elektroforezi (SDS-PAGE) ile yapılan protein analizleri ve GFAP antikoru ile yapılan immun blot analiz sonuçları gösterdi ki kullandığımız tümörlü dokularda GFAP'in sentezi mümkün görünmemektedir. Beyin dokusunda GFAP'in yeter miktarda bulunduğu gözlenmiştir. GFAP'in elde ettiğimiz tümör dokularında tespit edilememiş olması bu proteinin genellikle sinir sistemi dokularına spesifik olduğuna işaret etmektedir.

Anahtar Kelimeler: Glial fibriler asidik protein, tümör, köpek, tümör belirteçleri

Glial Fibrillary Acidic Protein (GFAP) is an intermediate filament protein in astrocytes of the Central Nervous System that has a molecular weight of approximately 50 kDa. Nearly 15 years ago, it has been acknowledged that GFAP can be used as a prototype antigen for nervous tissue identification and it has a standard marker value in basic and applied researches. As a member of cell skeleton proteins family, GFAP is thought to

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be important in regulating astrocyte motility. In case of a trauma, disease or a genetic disorder in central nervous system of vertebrates, astrocytes react in a way called Astrogliosis. Astrogliosis is characterised by rapid GFAP synthesis and identified by increased levels of this protein (6). A recent study in dogs revealed the fact that there is a link between GFAP expression and age. GFAP level in the dentate gyrus decreases and the morphology of astrocytes changes by years (9).

Cell surface antigens as well as monoclonal and polyclonal antibodies produced for cell skeleton proteins have a potential value in diagnosis of tumors (11). GFAP is a reliable marker for normal and glial associated neoplastic cells (2). Studies proved that the level of GFAP is closely related with the malignity of the astrocytoma cells (4). GFAP is extracted not only from a number of cells in central nervous system but also from some tumor tissues that are considered non-glial (5).

Extraction of GFAP has been determined in astrocytomas, glioblastomas, gliosarcomas, medulloblastomas, gangliogliomas, schwannomas, glial elemented teratomas, peripheral nerve sheath tumors, neuroectodermal tumors and ependymomas as well as cerebellar abiotrophies. Non-glial neoplasms, carcinomas, melanomas and sarcomas are negative for GFAP extraction (1, 2, 3, 7, 8, 12).

In scientific literatures it has been made clear that GFAP is a remarkable marker for the normal and neoplastic glial cells nevertheless there is no information about GFAP as a marker for the tumors not generally considered to be of glial origin. Therefore, the expression of GFAP in the several dog tumors except glial neoplasms was aimed to study.

14 tumor and control tissue samples were analysed in this study. The samples were histopathologically diagnosed by Ankara University, Faculty of Veterinary Medicine, Department of Pathology. All used samples were biopsy material which was obtained from pathology department. Diagnosed tissues as well as age, sex and race of the dogs are shown in Table 1.

Table 1: Neoplastic tissue samples of dogs used in this research
Tablo 1: *Bu araştırmada kullanılan neoplastik doku örnekleri*

Histopathologic Diagnose	Race	Age	Sex
Eosinophilic Granuloma	Terrier	6.5	Female
Malign Mixed Tumor	Terrier	10	Female
Malign Mixed Tumor	Terrier	16	Female
Leiomyoma	Terrier	9	Female
Complex Carcinoma	Terrier	10	Female
Sebaceous Gland Adenoma	Collie	5	Female
Mast Cell Tumor	Boxer	5	Male
Lenfoma	Pitbull	8	Male
Adenocarcinoma	Cocker	9	Female
Adenocarcinoma	Terrier	9	Female
Rhabdomyosarcoma	Kangal	7	Male
Lipoma	Kangal	7	Male
Malign Mixed Tumor	Terrier	12	Female
Malign Mixed Tumor	Terrier	11	Female

For the analysis of GFAP in histopathologically diagnosed neoplastic samples by SDS-PAGE, 20 µg tissue extracts were loaded on the polyacrylamide gels. Protein analysis of the samples was performed by using denaturing 10 % SDS-PAGE according to the method of Laemmli (10). SE 250-Mighty Small II Slab Gel Electrophoresis Unit (Hoefer Scientific Instruments) was used for electrophoretic analysis. Proteins in the gel were transferred to nitrocellulose membrane by using semi-dry transfer unit (Hoefer Scientific Instruments).

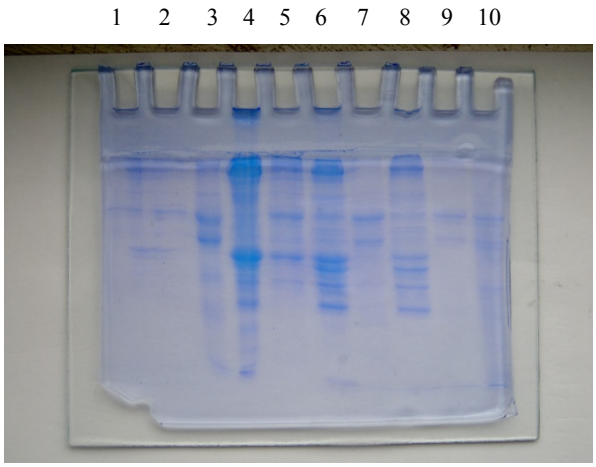

Figure 1a: Determination of protein profiles in normal and neoplastic tissues of dogs by SDS-PAGE analysis. Proteins of tumor tissues were seperated on 10 % polyacrylamide gel and stained with Coomassie Blue (G-250).

Lane 1; Sebaceous Gland Adenoma. Lane 2; Sebaceous Gland Adenoma Control. Lane 3; Mast Cell Tumor. Lane 4; Mast Cell Tumor Control, Lane 5; Leiomyoma. Lane 6; Leiomyoma Control. Lane 7; Rhabdomyosarcoma. Lane 8; Rhabdomyosarcoma Control. Lane 9; Lipoma. Lane 10; Lipoma Control

Şekil 1a: *Köpeklerin normal ve neoplastik dokularında SDS-PAGE analizi ile protein profillerinin belirlenmesi. Tümör doku proteinleri %10'luk poliakrilamid jelde ayrıştırıldı ve Coomassie mavisi (G-250) ile boyandı.*

Sıra 1; Sebaseöz Bez Adenomu. Sıra 2; Sebaseöz Bez Adenomu Kontrol. Sıra 3; Mast Hücreli Tümör. Sıra 4; Mast Hücreli Tümör Kontrol. Sıra 5; Leiomyom. Sıra 6; Leiomyom Kontrol. Sıra 7; Rhabdomyosarkom. Sıra 8; Rhabdomyosarkom Kontrol. Sıra 9; Lipom. Sıra 10; Lipom Kontrol.

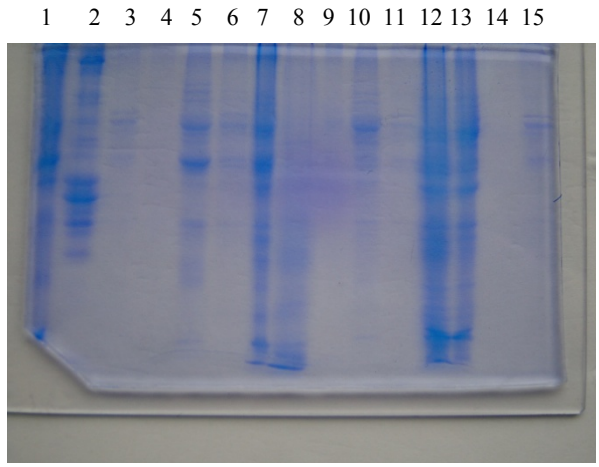

Figure 1b: Determination of protein profiles in normal and neoplastic tissues of dogs by SDS-PAGE analysis. Proteins of tumor tissues were seperated on 10 % polyacrylamide gel and stained with Coomassie Blue (G-250).

Lane 1; Eosinophilic Granuloma. Lane 2; Eosinophilic Granuloma Control. Lane 3; Mammary Glands Control. Lanes 4 and 5; Malign Mixed Tumor. Lane 6; Complex Carcinoma. Lanes 7 and 8; Adenocarcinoma. Lanes 9 and 10; Malign Mixed Tumor. Lane 11; Glandula mammaria Control. Lane 12; Lenfoma. Lane 13; Lenfoma Control. Lane 14; Brain Tissue Control. Lane 15; Mammary Glands Control

Şekil 1b: *Köpeklerin normal ve neoplastik dokularında SDS-PAGE analizi ile protein profillerinin belirlenmesi. Tümör doku proteinleri %10'luk poliakrilamid jelde ayrıştırıldı ve Coomassie mavisi (G-250) ile boyandı.*

Sıra 1; Eozinofilik Granüloma. Sıra 2; Eozinofilik Granüloma Kontrol. Sıra 3; Meme Dokusu Kontrol. Sıra 4; Malin Miks Tümör. Sıra 5; Malin Miks Tümör. Sıra 6; Kompleks Karsinom. Sıra 7; Adenokarsinom. Sıra 8; Adenokarsinom. Sıra 9; Malin Miks Tümör. Sıra 10; Malin Miks Tümör. Sıra 11; Meme Dokusu Kontrol. Sıra 12; Lenfoma. Sıra 13; Lenfoma Kontrol. Sıra 14; Beyin Dokusu Kontrol. Sıra 15; Meme Dokusu Kontrol.

Rabbit-anti-GFAP, as primary antibody, and Goat anti-rabbit-GFAP, as secondary antibody, were used in order to search antigens on the nitrocellulose membrane after semi-dry blotting (immunoblotting).

Profiles of the Coomassie Blue (G-250) dyed proteins are shown in Figure 1a and 1b. As shown in the figures, control and tumor tissues have different numbers of protein bands which means that control and tumor tissues have different protein contents from each other.

In dogs, GFAP activity is previously observed especially in fibrous astrocytes, Schwann cells, axons and peripheral ganglions (13). Therefore brain tissue was used as positive control in order to show if the immunostaining method we used is working with anti-GFAP (Figure 2a and 2b).

1 2 3 4 5 6 7 8 9 10

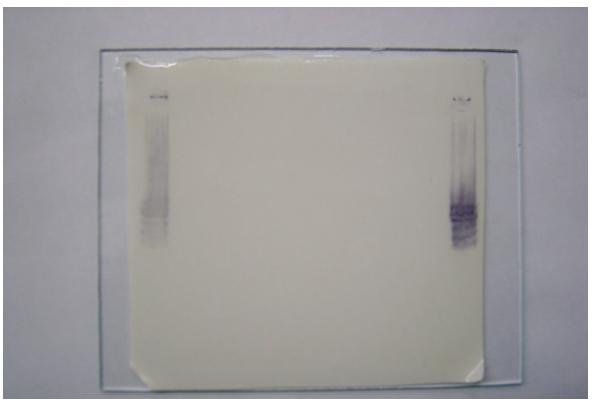

Figure 2a: Seperated tissue proteins by SDS-PAGE were transferred from gel to nitrocellulose membrane by semi-dry blotting and immunostained with GFAP antibody (immunoblotting).

Lane 1; Brain Tissue Control. Lane 2; Mast Cell Tumor. Lane 3; Mast Cell Tumor Control. Lane 4; Leiomyoma. Lane 5; Leiomyoma Control. Lane 6; Rhabdomyosarcoma. Lane 7; Rhabdomyosarcoma Control. Lane 8; Lipoma. Lane 9; Lipoma Control. Lane 10; Brain Tissue Control

Şekil 2a: *SDS-PAGE ile ayrıştırılan doku proteinleri yarı-kuru blot yöntemi ile jel'den nitroselüloz membrana aktarılmış ve GFAP antikorları ile immun boyama yapılmıştır (İmmun blot yöntemi).*

Sıra 1; Beyin Dokusu Kontrol. Sıra 2; Mast Hücreli Tümör. Sıra 3; Mast Hücreli Tümör Kontrol. Sıra 4; Leiomyom. Sıra 5; Leiomyom Kontrol. Sıra 6; Rhabdomyosarkom. Sıra 7; Rhabdomyosarkom Kontrol. Sıra 8; Lipom. Sıra 9; Lipom Kontrol. Sıra 10; Beyin Dokusu Kontrol.

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

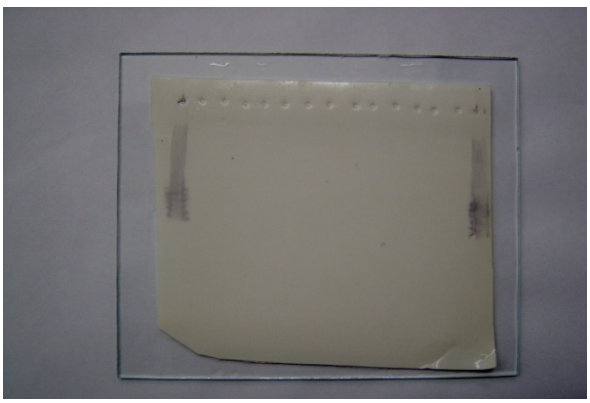

Figure 2b: Seperated tissue proteins by SDS-PAGE were transferred from gel to nitrocellulose membrane by semi-dry blotting and immunostained with GFAP antibody (immunoblotting).

Lane 1; Brain Tissue Control. Lane 2; Eosinophilic Granuloma. Lane 3; Eosinophilic Granuloma Control. Lane 4; Mammary Glands Control. Lanes 5-7; Malign Mixed Tumor. Lanes 8 and 9; Adenocarcinoma. Lane 10; Complex Carcinoma. Lane 11; Lenfoma. Lane 12; Lenfoma Control. Lane 13; Sebaceous Gland Adenoma. Lane 14; Sebaceous Gland Adenoma Control. Lane 15; Brain Tissue Control

Şekil 2b: *SDS-PAGE ile ayrıştırılan doku proteinleri yarı-kuru blot yöntemi ile jel'den nitroselüloz membrana aktarılmış ve GFAP antikorları ile immun boyama yapılmıştır (İmmun blot yöntemi).*

Sıra 1; Beyin Dokusu Kontrol. Sıra 2; Eozinofilik Granüloma. Sıra 3; Eozinofilik Granüloma Kontrol. Sıra 4; Meme Dokusu Kontrol. Sıra 5-7; Malin Miks Tümör. Sıra 8 ve 9; Adenokarsinom. Sıra 10; Kompleks Karsinom. Sıra 11; Lenfoma. Sıra 12; Lenfoma Kontrol. Sıra 13; Sebaseöz Bez Adenomu. Sıra 14; Sebaseöz Bez Adenomu Kontrol. Sıra 15; Beyin Dokusu Kontrol.

The results of SDS–PAGE and semi-dry blotting analysis showed that GFAP is not being expressed by the 14 tumors which we studied (See Figure 2a and Figure 2b). It has been observed that GFAP expression was seen in enough amounts as expected in the brain tissues. The fact that GFAP has not been identified in the tumor tissues we analysed points out that this protein is quite specific to nervous system tissues.

In conclusion, GFAP is not being expressed in neoplastic tissue samples of dogs with eosinophilic granuloma, malign mixed tumor, leiomyoma, complex carcinoma, sebaceous gland adenoma, mast cell tumor, lenfoma, adenocarcinoma, rhabdomyosarcoma and lipoma. These results suggest that GFAP cannot be used as a marker for nonglial tumor tissues in dogs. Obtained results can be considered as an original data in terms of non-glial origin tumors since GFAP is a specific marker protein to healthy and neoplastic glial cells.

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