Overexpression of the anti-apoptotic protein BAG3 in human choroidal melanoma: A case report

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Abstract. Bcl-2-associated athanogene 3 (BAG3), a co-chaperone of heat shock protein 70 (HSP70), exerts anti-apoptotic effects in various malignant tumors. However, relationships between choroidal melanoma and BAG3 are poorly studied. This study investigated the expression of BAG3 in a case of human choroidal melanoma. Funduscopy, computed tomography, and single-photon emission computed tomography with the intravenous injection of N-isopropyl-p-[123]I iodoamphetamine strongly indicated choroidal melanoma in a 68-year-old woman. Accordingly, we carried out an enucleation and pathological diagnosis. Proteins and total RNA were extracted from normal retinochoroidal and tumor tissues. Proteins were also extracted from ocular nevus tissues of other patients. We examined the expression of BAG3 protein and mRNA using Western blotting and the real-time quantitative polymerase chain reaction, respectively. Immunohistochemical stains were positive for melan-A, HMB-45, and S-100. Histopathology confirmed a choroidal melanoma. The expression of BAG3 protein and mRNA in the choroidal melanoma tissue was upregulated with respect to both normal retinochoroidal tissue and ocular nevus tissues from other patients. Because BAG3 may inhibit apoptosis of choroidal melanoma and facilitate its survival, overexpression of this gene product may be a prognostic marker and therapeutic target.

Introduction
Choroidal melanoma is the most common intraocular tumor in adults, and it metastasizes mainly to the liver (1). Poor prognosis is related to various clinical factors such as tumor size (2). Furthermore, various molecular factors are associated with poor prognosis (3,4).

Heat shock proteins (HSPs) function as molecular chaperones and exert cytoprotective effects. Among the HSPs, proteins from the HSP70 family play central roles as molecular chaperones. Bcl-2-associated athanogene 3 (BAG3) belongs to a family of co-chaperones that interacts with the ATPase domain of HSP70 (5). Although BAG3 is expressed weakly in normal cells, it is overexpressed in various malignant tumors (6-14). In melanoma cells, BAG3 is upregulated, and exerts cell survival and anti-apoptotic effects (15-17). However, relationships between choroidal melanoma and BAG3 are poorly studied. Therefore, we investigated the expression of BAG3 in human choroidal melanoma as compared to normal and ocular nevus tumor tissues.

Case report
Patients and clinical materials. A 68-year-old woman was referred to Toyama University Hospital for further evaluation of a left intraocular mass. Funduscopy revealed a pigmented choroidal mass in the temporal fundus of her left eye. B-Mode ultrasonography revealed a choroidal protrusion (Fig. 1A). Computed tomography revealed an enhanced intraocular mass (Fig. 1B). Single-photon emission computed tomography revealed a high accumulation of N-isopropyl-p-[123]I iodoamphetamine after its intravenous injection (Fig. 1C) (18).

To definitively treat this strongly suspected case of choroidal melanoma, we enucleated the eye. Immunohistochemical stains were positive for melan-A (Fig. 2), HMB-45, and S-100 (not shown) (19). Histopathology confirmed choroidal melanoma without vascular or optic nerve invasion. Additionally, we surgically resected a conjunctival tumor from a 44-year-old man (Fig. 3A) and a lid tumor from a 74-year-old man...
Normal retinochoroidal and melanoma tissue samples were obtained from the enucleated eye (Fig. 4), and nevus tissue samples were obtained from the resected tumor tissues (Fig. 3A and B). Our procedures conformed to the tenets of the World Medical Association's Declaration of Helsinki. Written informed consent was obtained from the patients after provision of sufficient information about the procedures.

Western blotting. Protein extracts were prepared by homogenizing tissue samples in a lysis buffer (150 mM NaCl, 1% Nonidet P-40, and 50 mM Tris-HCl, pH 8.0) containing a protease inhibitor cocktail (Nacalai Tesque, Kyoto, Japan). After electrophoresis on sodium dodecyl sulfate-polyacrylamide gels, proteins were transferred electrophoretically onto polyvinylidene fluoride membranes. The following primary antibodies were used: rabbit monoclonal anti-BAG3 (GTX62327; GeneTex Inc., Irvine, CA, USA); mouse monoclonal anti-HSP70 (SR-B810; MBL, Nagoya, Japan); rabbit monoclonal anti anti-HSF1 (GTX62022; GeneTex Inc.) and mouse monoclonal anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (MAB374; Millipore, Temecula, CA, USA). The immunoreactive proteins were visualized using a luminescence image analyzer (LAS 4000mini; GE Healthcare, Tokyo, Japan) with an enhanced chemiluminescence detection system. GAPDH served as the loading control.

RNA isolation. Using an RNeasy Total RNA Extraction kit (Qiagen K.K., Tokyo, Japan), total RNA was extracted from tissue samples and treated with on-column DNase I (RNase-free DNase kit, Qiagen K.K.) (20).

Quantitative polymerase chain reaction (qPCR). qPCR was performed on a Real-Time PCR Mx3005P system (Agilent Technologies, Santa Clara, CA, USA) using a SYBR PreMix ExTaq kit (Takara Bio, Inc., Shiga, Japan). The relevant primer sequences are listed in Table I. mRNA expression levels for each protein were normalized to the mRNA expression level for GAPDH (20).

Statistical analysis. Measurements are reported as means ± standard deviations. Student’s t-test was used for statistical analysis, and P<0.05 was considered statistically significant.

Results
To analyze the involvement of BAG3 within a choroidal melanoma, we examined its protein and mRNA expression levels using western blotting and qPCR, respectively. The BAG3 protein level in the human choroidal melanoma tissue was upregulated compared to that in normal retinochoroidal tissue (Fig. 5A). Furthermore, as observed using Western blotting, the expression levels of heat shock factor 1 (HSF1) and HSP70 were upregulated in human choroidal melanoma relative to expression levels in normal retinochoroidal tissues (Fig. 5A). Similarly, qPCR indicated that the BAG3 mRNA level in the human choroidal melanoma was significantly higher than that in normal retinochoroidal tissue (n=4, P=0.000291).
(Fig. 5B). Additionally, we confirmed BAG3 expression in choroidal melanoma using immunohistochemical analysis (Fig. 5C).

Western blots also indicated that the BAG3 level in the human choroidal melanoma tissue was upregulated compared to those in nevus tissue samples from other patients. Moreover, BAG3 levels in the conjunctival nevus were higher than those in the lid nevus (Fig. 6). These findings suggest that BAG3 was upregulated in the human choroidal melanoma relative to normal retinochoroidal and nevus tissues.

Discussion

The mechanisms of choroidal melanoma progression and metastasis remain poorly understood, and treatment options are limited. Regardless of the progress of diagnostic technology, choroidal melanoma causes death due to liver metastasis (21). Accordingly, the study of choroidal melanoma-specific biomarkers is important for improving prognosis accuracy. It is thought that an association between the heat shock response and melanoma is important. HSF1 is required for
melanoma invasion and metastasis (22). BAG3, a co-chaperone of HSP70, is overexpressed in multiple malignant tumors and exerts anti-apoptotic effects (15-17). Observations in vitro and in vivo indicate that the induction of BAG3 is at least partly mediated by the activation of HSF1 (23). In this study, BAG3 levels were upregulated via HSF1 activation in human choroidal melanoma relative to its expression levels in normal retinochoroidal tissues. However, little is known about the anti-apoptotic role of BAG3 in human choroidal melanoma.

To our knowledge, we are the first to report overexpression of BAG3 protein and mRNA in human choroidal melanoma relative to expression levels in normal retinochoroidal and ocular nevus tissues. Franco et al. reported that BAG3 levels in eye melanoma are relatively low, but are related to metastasis at other sites (15). It is possible that BAG3-positive choroidal melanoma is associated with a poor prognosis. We think that careful follow-up of patients is necessary in BAG3-positive choroidal melanoma.

Similar to its effects in other malignant tumors, BAG3 may contribute to survival through anti-apoptotic activity in choroidal melanoma. We believe that BAG3 may be a prognostic marker and therapeutic target. Further investigation is necessary to understand the relationships between choroidal melanoma and BAG3.

In conclusion, BAG3 is overexpressed in human choroidal melanoma relative to other related tissues. Our findings suggest that BAG3 may offer a therapeutic target for patients with choroidal melanoma.

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References

1. Wöll E, Bedikian A and Legha SS: Uveal melanoma: Natural history and treatment options for metastatic disease. Melanoma Res 9: 575-581, 1999.
2. Shields CL, Furuta M, Thangappan A, Nagori S, Mashayekhi A, Lally DR, Kelly CC, Rudich DS, Nagori AV, Wakade OA, et al: Metastasis of uveal melanoma millimeter-by-millimeter in 8033 consecutive eyes. Arch Ophthalmol 127: 989-998, 1999.
3. Mooy CM and De Jong PT: Prognostic parameters in uveal melanoma: A review. Surv Ophthalmol 41: 215-228, 1996.
4. Scholes AG, Damato BE, Nunn J, Hiscott P, Grieron J and Field JK: Monosomy 3 in uveal melanoma: Correlation with clinical and histologic predictors of survival. Invest Ophthalmol Vis Sci 44: 1008-1011, 2003.
5. Takayama S, Xie Z and Reed JC: An evolutionarily conserved family of Hsp70/Hsc70 molecular chaperone regulators. J Biol Chem 274: 781-786, 1999.
6. Liao Q, Ozawa F, Friess H, Zimmermann A, Takayama S, Reed JC, Kleej J and Büchler MW: The anti-apoptotic protein BAG-3 is overexpressed in pancreatic cancer and induced by heat stress in pancreatic cancer cell lines. FEBS Lett 503: 151-157, 2001.
7. Kassis JN, Virador VM, Guancial EA, Kimm D, Ho AS, Mishra M, Chuan EY, Cook J, Gius D and Kohn EC: Genomic and phenotypic analysis reveals a key role for CCNI (CYR61) in BAG3-modulated adhesion and invasion. J Pathol 218: 495-504, 2009.