

RESEARCH ARTICLE

Soluble CD30: A Possible Serum Tumor Marker for Primary Effusion Lymphoma

Manthana Michai^{1,2}, Hiroki Goto¹, Shinichiro Hattori¹, Kulthida Vaeteewoottacharn^{1,3}, Chaisiri Wongkham³, Sopit Wongkham³, Seiji Okada^{1*}

Abstract

Background: The serum level of soluble CD30 (sCD30) is known to be increased with several lymphomas and to correlate with prognosis. Primary effusion lymphoma (PEL) is a highly aggressive malignant lymphoma with poor prognosis, but the existence and significance of sCD30 in PEL have not yet been investigated in detail. **Objectives:** Since the membrane type of CD30 is frequently expressed on the surface of PEL cells, we compared the expression of the membrane type of CD30 and the production of sCD30 among PEL cell lines as well as other lymphomas. **Methods:** The expression of surface CD30 in various lymphoma cell lines was analyzed with flow cytometry and sCD30 was quantified by ELISA. **Results:** Both surface and sCD30 were detected on PEL cell lines as well as on Hodgkin's lymphoma and adult T-cell leukemia/lymphoma cell lines. Surface CD30 and sCD30 levels of each cell lines correlated with each other. **Conclusion:** The serum level of sCD30 appear to be a useful biological tumor marker for the diagnosis and management of CD30-positive PEL.

Keywords: Primary effusion lymphoma - soluble CD30 - HIV-1 - tumor marker - malignant lymphoma

Asian Pacific J Cancer Prev, 13 (10), 4939-4941

Introduction

CD30, a 120 kDa type I surface glycoprotein, is a member of the tumor necrosis factor receptor (TNFR) superfamily, which is consistently expressed by Hodgkin and Reed-Sternberg cells in Hodgkin's lymphoma (HL) and by neoplastic cells such as CD30+ anaplastic large-cell type lymphoma (ALCL) and human T-lymphotropic virus type 1 positive (HTLV-1+) adult T cell leukemia/lymphoma (ATLL). CD30 regulates their proliferation, differentiation and apoptotic cells death, depending on the cell type and developmental stage (Horie and Watanabe, 1998; Al-Shamkhani, 2004). The soluble form of CD30 (sCD30) is produced by metalloprotease cleavage of the juxta-membrane region, released as an extracellular region of 85 kDa protein. CD30-positive cells are known to release sCD30 *in vitro* and *in vivo* and it cannot be detected in the sera of healthy donors (Al-Shamkhani, 2004). Increased serum levels of this molecule have been reported in patients with CD30+ neoplasms such as HL (Nadali et al., 1994; Visco et al., 2006), ALCL (Nadali et al., 1995), and ATL (Higuchi et al., 2005; Nishioka et al., 2005). In most conditions, serum levels of sCD30 correlated with disease activity and/or prognosis.

Primary effusion lymphoma (PEL) is a highly aggressive lymphoma which was first proposed as a new disease entity in 1996 (Chen et al., 2007). PEL is

also known to express CD30 on the surface of tumor cells (Nador et al., 1996); however, the existence and significance of sCD30 have not yet been investigated.

In this study, we investigated the expression of surface CD30 and production of soluble CD30 in PEL cell lines. Our results suggest the usefulness of serum sCD30 as a biological tumor marker in PEL patients.

Materials and Methods

Cell lines

The human PEL cell line, BCBL-1, was obtained through the AIDS Research and Reference Reagent Program (Division of AIDS, NIAID, NIH), and TY-1 (Katano et al., 1999) and RM-P1 (Miyagi et al., 2002) were gifts from Dr. H. Katano (National Institute of Infectious Diseases, Japan) and Dr. T. Taira (University of the Ryukyus, Japan), respectively. BC-3 was purchased from ATCC. ATL cell lines, MT-2 and MT-4, were a gift from Prof. H. Mitsuya (Kumamoto University). HD cell lines, HD70, KM-H2 and L540, were a gift from Dr. Shinya Suzu (Kumamoto University). U937 and Raji were obtained from RIKEN cell bank (Tsukuba, Japan). The cell lines were maintained in RPMI1640 supplemented with 10% heat-inactivated fetal calf serum, penicillin (100 U/ml) and streptomycin (100 mg/ml) in a humidified incubator at 37°C and 5% CO₂.

¹Division of Hematopoiesis, Center for AIDS Research, Kumamoto University, Kumamoto, Japan, ²Department of Clinical Pathology, Khon Kaen Hospital, ³Department of Biochemistry, Faculty of Medicine, Khon Kaen University, Thailand *For correspondence: okadas@kumamoto-u.ac.jp

Detection of cell surface CD30 with Flow cytometry

The cells (1x10⁶/sample) were stained with APC conjugated mouse anti-human CD30 antibody (Biolegends, San Diego, CA). Isotype-matched control mAbs were used as a negative control. After 30-min incubation on ice in the dark, cells were washed twice with staining medium (3% FCS, 0.1% NaN₃ in PBS), re-suspended in staining medium with 1 mg/ml propidium iodide, and analyzed using an LSR II flow cytometer (BD Bioscience, San Jose, CA). Data were analyzed with FlowJo software (Tree Star, San Carlos, CA).

Detection of soluble CD30 (sCD30)

The cell lines (5x10⁵/ml) were cultured for 24 hrs and the level of sCD30 in culture supplements was determined using an enzyme linked immunoassay kit (Bender MedSystems, Vienna, Austria), according to the manufacturer's instructions. Briefly, micro-well strips pre-coated with monoclonal antibody against soluble CD30 were used. A 10-fold dilution of positive control material was prepared from reconstituted sCD30 standard supplied by the manufacturer. Anti-human horseradish peroxidase conjugate was used as the detection antibody. The plate was read using an ELISA plate reader (Multiskan, Thermo Electron Vantaa, Finland). and the optical density levels detected for each sample were used to estimate the sCD30 values off the standard curve.

Results

Surface expression of CD30 in lymphoma cell lines

Surface CD30 expression was determined in various lymphoma cell lines with flow cytometry. The HL and ATL cell lines expressed surface CD30 as expected. PEL cell lines also expressed surface CD30 with various expression levels. In contrast, U937 and Raji cell lines did not express cell surface CD30 (Figure 1).

Production of sCD30 from lymphoma cell lines

Next, sCD30 levels in culture supernatants were analyzed. After cell lines (5x10⁵ /ml) had been cultured for 24 hrs, the production of sCD30 was quantified by ELISA. As expected, PEL cell lines as well as HL and ATL cell

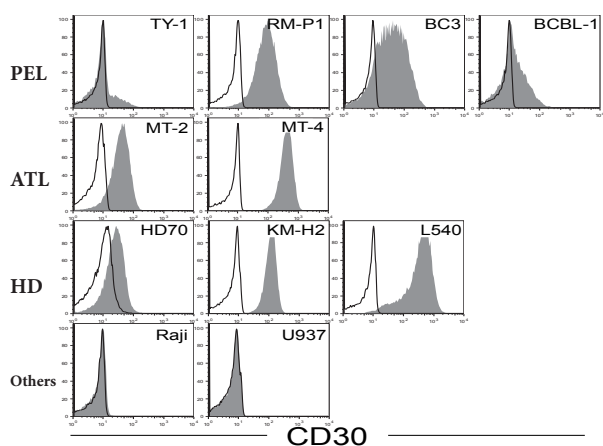


Figure 1. Surface CD30 Expression in Lymphoma Cell lines. Surface expression levels of CD30 on various lymphoma cell lines were analyzed with flow cytometry

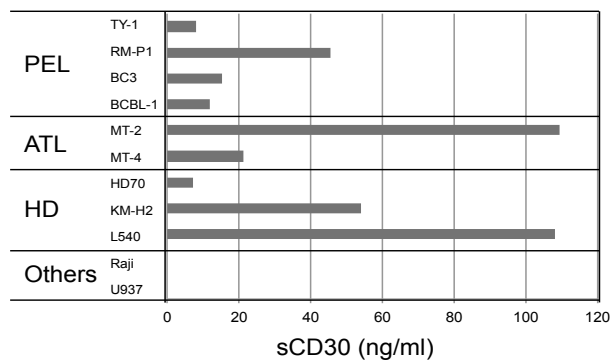


Figure 2. sCD30 Production by Lymphoma Cell Lines. The sCD30 levels of culture supernatants were measured with enzyme-linked immunosorbent assay

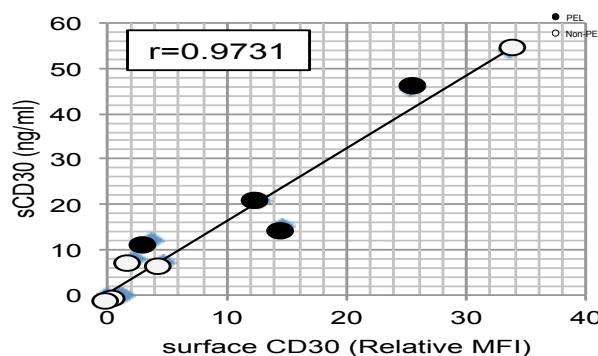


Figure 3. Correlation between Surface and sCD30 in PEL Cell Lines

lines produced significant amounts of sCD30. In contrast, U937 and Raji cell lines did not produce detectable levels of sCD30 (Figure 2).

Correlation of surface and soluble CD30 in lymphoma cell lines

Comparison between surface and sCD30 revealed that sCD30 production correlated with the surface expression of CD30 (r=0.9731) (Figure 3). These results suggest that soluble CD30 could be a tumor marker for primary effusion lymphoma.

Discussion

In the present study, we demonstrated that PEL cells produced sCD30 as well as the surface expression of CD30, and the levels of sCD30 correlated with the surface expression of CD30. Since sCD30 is expected to be a useful diagnostic and prognostic marker of lymphoma (Purdue et al., 2009; Ambinder et al., 2010; Vermeulen et al., 2011), it is also expected to be a useful tumor marker for PEL.

CD30 was originally identified as a cell surface antigen on Hodgkin and Reed Sternberg cells by the monoclonal antibody Ki-1. CD30 has also been detected on the surface of activated T and B cells, Epstein-Barr virus (EBV) transformed cells, HTLV-1 transformed cells, and some non-Hodgkin lymphoma (NHL). Like many other receptors, CD30 is shed in the serum of many individuals and can reach high levels in patients with HL and CD30-positive lymphoma (Nadali et al., 1994). An elevated level of serum CD30 in these patients has been reported to confer poor prognosis, perhaps because it reflects tumor

burden (Al-Shamkhani, 2004).

PEL is a human herpes virus-8 (HHV-8)-associated and rare type of lymphoma usually confined to the body cavity and commonly observed in HIV-1 infected patients (Chen et al., 2007). PEL cells usually express CD30 on the cell surface (Nador et al., 1996); however, the production of sCD30 has not yet been investigated. We show here that PEL cells also produce sCD30. Although its physiologic effects, if any, are unknown, it is suggested that sCD30 could be a useful tumor marker in PEL as well as other NHL and HL. In addition, since the serum level of sCD30 is associated with an increased risk of both AIDS-related and un-related lymphoma (Breen et al., 2006; Purdue et al., 2009; Ambinder et al., 2010; Jarrin et al., 2011; Vermeulen et al., 2011), monitoring of serum-soluble CD30 is expected to predict the occurrence of lymphoma, including PEL. It is of interest that although most B-cell NHL does not express CD30 on the surface, the serum level of sCD30 is increased (Purdue et al., 2009). This is because sCD30 is generated from both activated B cells and the immune microenvironment conducive to chronic B cell stimulation (Jarrin et al., 2011; Vermeulen et al., 2011).

In this study, we used PEL cell lines instead of primary PEL cells because the number of AIDS-related lymphoma patients is still low and PEL is extremely rare in Japan (Nagai et al., 2008). In addition, PEL cell lines maintain the features of primary PEL cells and are frequently used as a model of PEL (Drexler et al., 1998). Further study is needed to confirm these findings in other patients.

In conclusion, the present study clarified that sCD30 is produced from PEL cell lines. These results indicate that sCD30 can be used as a marker to predict and diagnose PEL.

Acknowledgements

We thank Ms. I. Suzu for technical assistance and Ms. K. Tokunaga for secretarial assistance. This work was supported in part by a Health and Labour Sciences Research Grant from the Ministry of Health, Labour, and Welfare of Japan (H22-AIDS-I-002), by the Global COE program "Global Education and Research Center Aiming at the Control of AIDS" from the Ministry of Education, Science, Sports, and Culture of Japan, by Tokyo Biochemical Research Foundation Postdoctoral Fellowship for Asian Researchers, and by the RONPAKU program from the Japan Society for the Promotion of Science (JSPS). Conflict of interest, the authors declare that there are no conflicts of interest in this study.

References

- Al-Shamkhani A (2004). The role of CD30 in the pathogenesis of haematopoietic malignancies. *Curr Opin Pharmacol*, **4**, 355-9.
- Ambinder RF, Bhatia K, Martinez-Maza O, et al (2010). Cancer biomarkers in HIV patients. *Curr Opin HIV AIDS*, **5**, 531-7.
- Breen EC, Fatahi S, Epeldegui M, et al (2006). Elevated serum soluble CD30 precedes the development of AIDS-associated non-Hodgkin's B cell lymphoma. *Tumour Biol*, **27**, 187-94.
- Chen YB, Rahemtullah A, Hochberg E (2007). Primary effusion

- lymphoma. *Oncol*, **12**, 569-76.
- Drexler HG, Uphoff CC, Gaidano G, et al (1998). Lymphoma cell lines: *in vitro* models for the study of HHV-8+ primary effusion lymphomas (body cavity-based lymphomas). *Leukemia*, **12**, 1507-17.
- Higuchi M, Matsuda T, Mori N, et al (2005). Elevated expression of CD30 in adult T-cell leukemia cell lines: possible role in constitutive NF-kappaB activation. *Retrovirology*, **2**, 29.
- Horie R, Watanabe T (1998). CD30: expression and function in health and disease. *Semin Immunol*, **10**, 457-70.
- Jarrin I, Boval B, Mazon MC, et al (2011). Can serum soluble CD23 OR CD30 predict the occurrence of lymphoma in HIV-infected patients? *J Acquir Immune Defic Syndr*, **57**, 58-61.
- Katano H, Hoshino Y, Morishita Y, et al (1999). Establishing and characterizing a CD30-positive cell line harboring HHV-8 from a primary effusion lymphoma. *J Med Virol*, **58**, 394-401.
- Miyagi J, Masuda M, Takasu N, et al (2002). Establishment of a primary effusion lymphoma cell line (RM-P1) and *in vivo* growth system using SCID mice. *Int J Hematol*, **76**, 165-72.
- Nadali G, Vinante F, Ambrosetti A, et al (1994). Serum levels of soluble CD30 are elevated in the majority of untreated patients with Hodgkin's disease and correlate with clinical features and prognosis. *J Clin Oncol*, **12**, 793-7.
- Nadali G, Vinante F, Stein H, et al (1995). Serum levels of the soluble form of CD30 molecule as a tumor marker in CD30+ anaplastic large-cell lymphoma. *J Clin Oncol*, **13**, 1355-60.
- Nador RG, Cesarman E, Chadburn A, et al (1996). Primary effusion lymphoma: a distinct clinicopathologic entity associated with the Kaposi's sarcoma-associated herpes virus. *Blood*, **88**, 645-56.
- Nagai H, Iwasaki N, Odawara T, et al (2008). Actual status of AIDS-related lymphoma management in Japan. *Int J Hematol*, **87**, 442-3.
- Nishioka C, Takemoto S, Kataoka S, et al (2005). Serum level of soluble CD30 correlates with the aggressiveness of adult T-cell leukemia/lymphoma. *Cancer Sci*, **96**, 810-5.
- Purdue MP, Lan Q, Martinez-Maza O, et al (2009). A prospective study of serum soluble CD30 concentration and risk of non-Hodgkin lymphoma. *Blood*, **114**, 2730-2.
- Vermeulen R, Hosnijeh FS, Portengen L, et al (2011). Circulating soluble CD30 and future risk of lymphoma; evidence from two prospective studies in the general population. *Cancer Epidemiol Biomarkers Prev*, **20**, 1925-7.
- Visco C, Nadali G, Vassilakopoulos TP, et al (2006). Very high levels of soluble CD30 recognize the patients with classical Hodgkin's lymphoma retaining a very poor prognosis. *Eur J Haematol*, **77**, 387-94.