RESEARCH ARTICLE

Expression of the Class II and III Beta-Tubulin in Neoplastic and Non-Neoplastic Lymphoid Tissues

Nor Syahida Binti Yusof, Fereshteh Ameli, Chandramaya Sabrina Florence, Muaatamarulain Mustangin, Faridah Abd Rahman, Noraidah Masir*

Abstract

Aim: Abnormal expression patterns of beta-tubulin isotypes may provide a molecular rationale for the behaviour of lymphoma subtypes. In the present study class II and III beta-tubulin expression was assessed in non-neoplastic and neoplastic lymphoid tissues with reference to potential utility as new tumour biomarkers. Methods and results: In this cross-sectional study class II and III beta-tubulin expression was assessed in 304 neoplastic and 20 normal lymphoid tissues using qualitative and semi-quantitative immunohistochemistry. Class II beta-tubulin was found to be positive in the germinal centres, mantle zone and interfollicular regions of normal lymphoid tissues. It was also expressed in 15/15 (100%) lymphoblastic lymphomas, 229/231 (99%) mature B cell lymphomas, 22/22 (100%) T/NK-cell lymphomas and 36/36 (100%) classical Hodgkin lymphomas. Class III beta-tubulin in contrast was germinal centre restricted and more selective, being found mainly in classical Hodgkin lymphomas (34/36 (94%)). It was also expressed in 58/171 (34%) DLBCL, 11/12 (92%) mantle cell lymphomas and 6/6 (100%) Burkitt lymphomas. Other mature B cell, T/NK cell lymphomas and precursor lymphoblastic lymphomas were usually negative. Conclusions: Class II beta-tubulin shows ubiquitous expression in neoplastic and non-neoplastic lymphoid tissues. In contrast, Class III beta-tubulin is germinal centre-restricted. Its consistent expression in classical Hodgkin lymphomas may point to use in the identification of Reed-Sternberg and Hodgkin cells. Its expression in a proportion of DLBCL, Burkitt and mantle cell lymphomas is of interest as this may be related to their aggressiveness.

Keywords: lymphoid lesion- lymphoma- immunohistochemistry

Introduction

Lymphomas comprise nearly 50% of hematological neoplasms with higher prevalence in developed countries (Roman and Smith, 2011; Siegel et al., 2014) and with one third of estimated cases occurring in Asia (Jemal et al., 2011). Due to the diversity in their prognosis, accurate classification of lymphoma subtype is essential (Harris et al., 2000; van Dongen and Orfao, 2012). This is based on clinical, morphological, immunophenotype and genetic features. However in some cases the immunophenotyping may lack precision (Campo et al., 2011). This warrants the identification of novel markers and recently the differential expression of Beta-tubulin subclasses has been studied for this purpose (Parker et al., 2014).

Microtubules are dynamic filamentous structures composed of Alpha and Beta- tubulin heterodimers that are important in cell proliferation, intracellular trafficking, signalling and migration in eukaryotic cells (Dumontet C, 2010). Diverse changes in the microtubule network have been identified and characterized in a wide variety of cancers. These changes are often associated with chemotherapy resistance and poor outcome (Parker et al., 2014). Class III Beta-tubulin over expression is associated with poor prognosis in epithelial cancers and non-Hodgkin lymphoma (Choi et al., 2012; Roque et al., 2013; Parker et al., 2014; Roque et al., 2014) while Class II, IVa and IVb Beta-tubulin are associated with non-small cell lung cancer, neuroblastoma, breast cancer and acute lymphoblastic leukemia (Verrills et al., 2003; Don et al., 2004; Cucchiarelli et al., 2008; Gan and Kavallaris, 2008; Gan et al., 2011; Lobert et al., 2011).

Although the association between Class II/III Beta-tubulins and chemotherapy resistance has been reported in certain lymphomas, there is a scarcity of data on their expression in non-neoplastic lymphoid tissues and other lymphoma subtypes (Verrills et al., 2003; Yoon et al., 2010; Choi et al., 2012). The aim of this study was to assess Class II and III Beta-tubulin expression in a wide variety of neoplastic and non-neoplastic lymphoid tissues.

Materials and Methods

This study was approved by the Universiti Kebangsaan Malaysia, Faculty of Medicine, Department of Pathology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia. *For Correspondence: noraidah@ppukm.ukm.edu.my
Tissue samples
Formalin-fixed paraffin-embedded tissue samples were obtained from 15 cases of precursor lymphoid neoplasms (nine B lymphoblastic lymphomas and six T lymphoblastic lymphomas), 171 diffuse large B cell lymphoma (DLBCL), 60 mature small B cell lymphomas, 22 T/Natural killer (NK) cell lymphomas including eight peripheral T cell lymphomas, five extranodal NK/T cell lymphomas, nasal type, four angioimmunoblastic T cell lymphomas, three subcutaneous panniculitis-like T cell lymphomas and two ALK- negative anaplastic large cell lymphomas) and 36 classical Hodgkin lymphomas (Table 1). Similarly, formalin-fixed, paraffin-embedded tissues of 20 non-neoplastic lymphoid tissues (lymph node = 4, tonsil = 4, spleen = 4, adenoid = 3, appendix = 3, ileum = 1, thymus = 1) were retrieved, along with their corresponding H&E stained slides.

Tissue preparation
Tissue microarrays were constructed using a tissue microarrayer (Alphelys MTA Booster, Plaisir France) for lymphoma cases where duplicated 1 mm tissue cores were taken from each tumour. Three µm thick whole paraffin sections were prepared from the 20 non-neoplastic lymphoid tissues.

Antibodies
Rabbit polyclonal antibody against Class II Beta-tubulin (Cat. No. ab103667, Abcam England) was used at a dilution of 1:50 with normal human brain tissue as the positive control. Rabbit monoclonal [EP1569Y] antibody against Class III Beta-tubulin (Cat. No. ab52623, Abcam) was used at a dilution of 1:100 with human breast carcinoma as the positive control.

Immunohistochemistry
Immunohistochemical staining was performed on the tissue microarray (TMA) sections and paraffin sections using the protocol from EnVision™ FLEX Mini Kit, High pH (Dako Denmark).

Immunostaining analysis
The staining for Class II Beta-tubulin and Class III Beta-tubulin was scored quantitatively and qualitatively at x400 magnification.

Staining was considered positive when cytoplasmic labelling was observed in more than 10% of tumour cells with equal or greater intensity to the internal positive controls. The absolute number of positive cells was also counted. Results were categorised into four groups as shown in Table 2. For Hodgkin and Reed-Sternberg (HRS) cells, expression was interpreted as positive when cytoplasmic membrane staining or at least paranuclear dot-like accentuation was observed in any number.

Statistical analysis
Data analysis was performed using the statistical package for social sciences (SPSS) version 19.0 (IBM Inc. Chicago, IL, USA). Pearson chi-square test was used to determine the association between different variables. P values of <0.05 were considered statistically significant.

Results
Normal lymphoid tissues
Class II Beta-tubulin
Class II Beta tubulin expression was observed in the follicles and interfollicular region. Germinal centre cells were strongly stained while cells in the mantle zone show a weaker expression (Figure 1A). It was also observed that scattered large cells in the interfollicular region were strongly positive while the smaller lymphocytes were weakly labelled (Figure 1B).

Class III Beta-tubulin
The expression of Class III Beta-tubulin was restricted to the germinal centre, staining predominantly the larger cells (Figure 1C) but not the smaller centrocytes. Cells in the mantle zone and the interfollicular region were negative.

The pattern of immunoreactivity for Class II and Class III Beta-tubulin is cytoplasmic and membrane associated. In addition to the lymphoid cells, their expression was also observed in the endothelial cells of blood vessels (Figure 1D).

Neoplastic lymphoid tissues
A total of 304 cases (consisting 164 males and 140 females) were included in the study. The lymphoma subtypes and the number of cases are presented in Table 1. Tissue samples
Formalin-fixed paraffin-embedded tissue samples were obtained from 15 cases of precursor lymphoid neoplasms (nine B lymphoblastic lymphomas and six T lymphoblastic lymphomas), 171 diffuse large B cell lymphoma (DLBCL), 60 mature small B cell lymphomas, 22 T/Natural killer (NK) cell lymphomas including eight peripheral T cell lymphomas, five extranodal NK/T cell lymphomas, nasal type, four angioimmunoblastic T cell lymphomas, three subcutaneous panniculitis-like T cell lymphomas and two ALK- negative anaplastic large cell lymphomas) and 36 classical Hodgkin lymphomas (Table 1). Similarly, formalin-fixed, paraffin-embedded tissues of 20 non-neoplastic lymphoid tissues (lymph node = 4, tonsil = 4, spleen = 4, adenoid = 3, appendix = 3, ileum = 1, thymus = 1) were retrieved, along with their corresponding H&E stained slides.

Tissue preparation
Tissue microarrays were constructed using a tissue microarrayer (Alphelys MTA Booster, Plaisir France) for lymphoma cases where duplicated 1 mm tissue cores were taken from each tumour. Three µm thick whole paraffin sections were prepared from the 20 non-neoplastic lymphoid tissues.

Antibodies
Rabbit polyclonal antibody against Class II Beta-tubulin (Cat. No. ab103667, Abcam England) was used at a dilution of 1:50 with normal human brain tissue as the positive control. Rabbit monoclonal [EP1569Y] antibody against Class III Beta-tubulin (Cat. No. ab52623, Abcam) was used at a dilution of 1:100 with human breast carcinoma as the positive control.

Immunohistochemistry
Immunohistochemical staining was performed on the tissue microarray (TMA) sections and paraffin sections using the protocol from EnVision™ FLEX Mini Kit, High pH (Dako Denmark).

Immunostaining analysis
The staining for Class II Beta-tubulin and Class III Beta-tubulin was scored quantitatively and qualitatively at x400 magnification.

Staining was considered positive when cytoplasmic labelling was observed in more than 10% of tumour cells with equal or greater intensity to the internal positive controls. The absolute number of positive cells was also counted. Results were categorised into four groups as shown in Table 2. For Hodgkin and Reed-Sternberg (HRS) cells, expression was interpreted as positive when cytoplasmic membrane staining or at least paranuclear dot-like accentuation was observed in any number.

Statistical analysis
Data analysis was performed using the statistical package for social sciences (SPSS) version 19.0 (IBM Inc. Chicago, IL, USA). Pearson chi-square test was used to determine the association between different variables. P values of <0.05 were considered statistically significant.

Results
Normal lymphoid tissues
Class II Beta-tubulin
Class II Beta tubulin expression was observed in the follicles and interfollicular region. Germinal centre cells were strongly stained while cells in the mantle zone show a weaker expression (Figure 1A). It was also observed that scattered large cells in the interfollicular region were strongly positive while the smaller lymphocytes were weakly labelled (Figure 1B).

Class III Beta-tubulin
The expression of Class III Beta-tubulin was restricted to the germinal centre, staining predominantly the larger cells (Figure 1C) but not the smaller centrocytes. Cells in the mantle zone and the interfollicular region were negative.

The pattern of immunoreactivity for Class II and Class III Beta-tubulin is cytoplasmic and membrane associated. In addition to the lymphoid cells, their expression was also observed in the endothelial cells of blood vessels (Figure 1D).

Neoplastic lymphoid tissues
A total of 304 cases (consisting 164 males and 140 females) were included in the study. The lymphoma subtypes and the number of cases are presented in Table 1.
Beta Tubulin Protein in Neoplastic and Non-Neoplastic Lymphoid Tissue

It can be summarised that almost all DLBCL were positive for Class II Beta-tubulin but only a proportion of cases were positive for Class III Beta-tubulin and when positive for the latter, they are usually in group 1 or 2 where the staining is heterogenous and low (Figure 2A). Burkitt Lymphoma

All six cases of Burkitt lymphomas were positive for class II (strong expression) and class III Beta-tubulin with the latter showing a spectrum of staining intensity (Table 3 and 4).

Diffuse Large B Cell Lymphoma

Class II Beta-tubulin was expressed in 169/171 cases (99%) where almost all positive cases were in groups 3 and 4 (Table 3) i.e., exhibiting high percentage of positivity. The two negative cases showed weak cytoplasmic staining in less than 10% of tumour cells.

Class III Beta tubulin

In contrast to Class II Beta-tubulin, the expression of Class III Beta-tubulin in DLBCL is more heterogeneous. It is positive in only 58/171 cases (36%) with a spectrum of positivity ranging from group 1 to group 4 (Table 4). The majority of positive cases (47/58, 81%) were in group 1 or group 2, in which less than 50% of cells were stained. Subgrouping according to the cell of origin (Sabattini et al., 2010) was possible in 37/58 cases. Of these, 7/58 (12%) positive cases were germinal centre B cell-like (GCB) subtype while 30/58 (52%) cases were non-GCB subtype.

It can be summarised that almost all DLBCL were positive for Class II Beta-tubulin but only a proportion of cases were positive for Class III Beta-tubulin and when positive for the latter, they are usually in group 1 or 2 where the staining is heterogenous and low (Figure 2A).

Burkitt Lymphoma

All six cases of Burkitt lymphomas were positive for class II (strong expression) and class III Beta-tubulin with the latter showing a spectrum of staining intensity (Table 3 and 4).

Small B Cell Lymphomas

Class II Beta-tubulin

Class II Beta-tubulin was expressed in 169/171 cases (99%) where almost all positive cases were in groups 3 and 4 (Table 3) i.e., exhibiting high percentage of positivity. The two negative cases showed weak cytoplasmic staining in less than 10% of tumour cells.

Class III Beta-tubulin

In contrast to Class II Beta-tubulin, the expression of Class III Beta-tubulin in DLBCL is more heterogeneous. It is positive in only 58/171 cases (36%) with a spectrum of positivity ranging from group 1 to group 4 (Table 4). The majority of positive cases (47/58, 81%) were in group 1 or group 2, in which less than 50% of cells were stained. Subgrouping according to the cell of origin (Sabattini et al., 2010) was possible in 37/58 cases. Of these, 7/58 (12%) positive cases were germinal centre B cell-like (GCB) subtype while 30/58 (52%) cases were non-GCB subtype.

It can be summarised that almost all DLBCL were positive for Class II Beta-tubulin but only a proportion of cases were positive for Class III Beta-tubulin and when positive for the latter, they are usually in group 1 or 2 where the staining is heterogenous and low (Figure 2A).
lymphoma (n=4) and ALK-negative anaplastic large cell lymphoma (n=2) (Table 3).

Class III Beta-tubulin

Class III beta-tubulin was expressed in 1/8 (13%) of peripheral T-cell lymphomas, 1/5 (20%) of NK/T cell lymphomas, 3/4 (75%) of angioimmunoblastic T-cell lymphoma, 3/3 (100%) of subcutaneous panniculitis-like T-cell lymphoma and 1/2 (50%) ALK-negative anaplastic large cell lymphoma (Table 4).

Lymphoblastic Lymphoma

Class II Beta-tubulin was expressed in all 15 cases of lymphoblastic lymphoma (9 B-ALL and 6 T-ALL) (Table 3).

Class III Beta-tubulin

Class II Beta-tubulin was expressed in all 36 classical Hodgkin lymphomas. It labels Hodgkin and Reed-Sternberg cells (HRS cells) as well background lymphocytes but the staining intensity was stronger in the HRS cells (Table 3).

Class III Beta-tubulin

Interestingly, almost all cases (34/36, 94%) of Hodgkin lymphoma were also positive for Class III Beta-tubulin showing a spectrum of positivity (Table 4). The labelling was seen in the cytoplasm of HRS cells with para-nuclear dot-like accentuation while the background lymphoid cells were negative (Fig.2B). This consistently positive pattern is not seen in the other lymphoma types with Class III Beta-tubulin.

Discussion

The present study revealed that classII Beta-tubulin has widespread expression in all cellular compartments of the lymphoid tissue including the germinal centre, the mantle zone and interfollicular regions. In addition, in the interfollicular region, two population of positivity was observed i.e., strongly positive scattered large cells and more weakly stained smaller lymphocytes. It would be of interest to characterise further the nature of these strongly positive large cells in this region as it may be related to the interfollicular large B cells(Marafioti et al., 2003) and DLBCL with unusual pattern(Haycocks and Zhao, 2010). The expression of class III Beta-tubulin in lymphoid tissues was more unique. It was found to be strictly restricted to the large cells in the germinal centres exhibiting cytoplasmatic and membrane associated labelling whilst cells in other compartments are negative. These observations highlight the potential use of class III Beta-tubulin as an additional marker for germinal centre

---

Nor Syahida Binti Yusof et al
Asian Pacific Journal of Cancer Prevention, Vol 18
1048 Asian Pacific Journal of Cancer Prevention, Vol 18
Our results revealed that Class III Beta-tubulin upregulation is associated with resistance to Vinca alkaloids or taxanes (Choi et al., 2012). Of interest is the expression of Class III Beta-tubulin in classical Hodgkin lymphoma. In contrast to the previous report (Choi et al., 2012) our results revealed that Class III Beta-tubulin is consistently expressed in the HRS cells, albeit with some degree of heterogeneity. Although the staining was heterogeneous, it was not difficult to identify the positive cells given that the surrounding lymphocytes were negative. The findings of the present study suggest that Class III Beta tubulin could be potentially used as an additional marker for Hodgkin and Reed Sternberg cells.
Nor Syahida Binti Yusof et al

in classical Hodgkin lymphoma together with CD30, CD15 and Pax5.

The findings that there were more Non-GCB DLBCL cases positive for Class III Beta-tubulin does not fit into our theory that positive tumours are usually germinal centre associated neither is it in keeping with previous report (Yoon et al., 2010). One possible explanation is the number of cases that could be classified as GCB and non-GCB (37/58) were small. Furthermore we know that in our own population (Rahman et al., 2013), most DLBCL cases are non-GCB subtype further skewing the distribution of the two subgroups.

It can be concluded that the unrestricted Class II Beta-tubulin expression renders it impractical as a marker of differentiation in lymphoma. In contrast, Class III Beta-tubulin expression is confined primarily to the germinal centre cells of normal lymphoid tissues and is more frequently expressed in aggressive lymphoma (DLBCL, BL and MCL). Further investigations are warranted to clarify the predictive role of Class III Beta-tubulin in lymphoid neoplasms. In classical Hodgkin lymphoma, Class III Beta-tubulin shows selective expression in the neoplastic cells. Therefore, we postulate that Class III Beta-tubulin might be potentially useful as an additional marker for Hodgkin and Reed Sternberg cells.

Conflict of interest statement

The authors whose names are listed above certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers’ bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Acknowledgements

This study was funded by the Faculty of Medicine, Universiti Kebangsaan Malaysia (project code: FF-207-2013).

References

Campos E, Swerdlow SH, Harris NL, et al (2011). The 2008 WHO classification of lymphoid neoplasms and beyond: evolving concepts and practical applications. Blood, 117, 5019-32.

Choi J-W, Lee J-H, Kim Y-S (2012). Expression of β-tubulin isotypes in classical Hodgkin's lymphoma. Pathol Int, 62, 287-90.

Cucchiarelli V, Hiser L, Smith H, et al (2008). Beta-tubulin isotype classes II and V expression patterns in non-small cell lung carcinomas. Cell Motil Cytoskeleton, 65, 675-85.

Don S, Verrills NM, Liaw TYE, et al (2004). Neuronal-associated microtubule proteins class III beta-tubulin and MAP2c in neuroblastoma: role in resistance to microtubule-targeted drugs. Mol Cancer Ther, 3, 1137-46.

Dumontet C JM (2010). Microtubule-binding agents: a dynamic field of cancer therapeutics. Nat Rev Drug Discov, 9, 790-803.

Gan PP, Kavalleris M (2008). Tubulin-targeted drug action: functional significance of class ii and class IV beta-tubulin in vinca alkaloid sensitivity. Cancer Res, 68, 9817-24.

Gan PP, McCarroll JA, Byrne FL, et al (2011). Specific β-tubulin isotypes can functionally enhance or diminish epothilone B sensitivity in non-small cell lung cancer cells. PLoS One, 6, e21717.

Harris NL, Jaffe ES, Diebold J, et al (2000). Lymphoma classification – from controversy to consensus: The R.E.A.L. and WHO Classification of lymphoid neoplasms. Ann Oncol, 11, 3-10.

Haycocks NG, Zhao XF (2010). Large B-cell lymphoma with an unusual infiltrating pattern: report of two cases. Int J Clin Exp Pathol, 3, 815-21.

Jemal A, Bray F, Center MM, et al (2011). Global cancer statistics. CA Cancer J Clin, 61, 69-90.

Lobert S, Jefferson B, Morris K (2011). Regulation of β-tubulin isotypes by micro-RNA 100 in MCF7 breast cancer cells. Cytokeleton (Hoboken), 68, 355-62.

Marafioti T, Jones M, Facchetti F, et al (2003). Phenotype and genotype of interfollicular large B cells, a subpopulation of lymphocytes often with dendritic morphology. Blood, 102, 2868-76.

Parker AL, Kavallaris M, McCarroll JA (2014). Microtubules and their role in cellular stress in cancer. Front Oncol, 4

Piccaluga PP, Agostinelli C, Calilano A, et al (2007). Gene expression analysis of angioimmunoblastic lymphoma indicates derivation from T follicular helper cells and vascular endothelial growth factor. Cancer Res, 67, 10703-10.

Roman E, Smith AG (2011). Epidemiology of lymphomas. Histopathology, 58, 4-14.

Roque DM, Bellone S, English DP, et al (2013). Tubulin-β-III overexpression by uterine serous carcinomas is a marker for poor overall survival after platinum/taxane chemotherapy and sensitivity to epothilones: Tubulin-β-III in Uterine Serous Carcinomas. Cancer, 119, 2582-92.

Roque DM, Buza N, Glasgow M, et al (2014). Class III β-tubulin overexpression within the tumor microenvironment is a prognostic biomarker for poor overall survival in ovarian cancer patients treated with neoadjuvant carboplatin/paclitaxel. Clin Exp Metastasis, 31, 101-10.

Sabattini E, Bacci F, Sagramoso C, et al (2010). WHO classification of tumours of haematopoietic and lymphoid tissues in 2008: an overview. Pathologica, 102, 83-7.

Sève P, Mackey J, Isaac S, et al (2005). Class III β-tubulin expression in tumor cells predicts response and outcome in patients with non–small cell lung cancer receiving paclitaxel. Mol Cancer Ther, 4, 2001-7.

Siegel R, Ma J, Zou Z, et al (2014). Cancer statistics, 2014. CA Cancer J Clin, 64, 9-29.

van Dongen JJM, Orfao A (2012). EuroFlow: resetting leukemia and lymphoma immunophenotyping. Basis for companion diagnostics and personalized medicine. Leukemia, 26, 1899-907.

Verrills NM, Walsh BJ, Cobon GS, et al (2003). Proteome analysis of vinca alkaloid response and resistance in acute lymphoblastic leukemia reveals novel cytoskeletal alterations. J Biol Chem, 278, 45082-93.

Yoon SO, Kim WY, Go H, et al (2010). Class III β-tubulin shows unique expression patterns in a variety of neoplastic and non-neoplastic lymphoproliferative disorders. Am J Surg Pathol, 34, 645-55.