Research Article

Stem Cell & Regenerative Medicine

Human c-Cbl and Cbl-b Proteins are More Highly Expressed in the Thymus Compared to the Testis

Mazo Koné*, Rachida Salah and Harir Noria

Department of Biology, Faculty of Science, Djillali Liabbes University, Sidi Bel Abbes, Algeria.

*Correspondence:
Mazo Koné, Department of Biology, Faculty of Science, Djillali Liabbes University, Sidi Bel Abbes, Algeria, E-mail: mzoalger@yahoo.fr.

Received: 12 November 2017; Accepted: 14 December 2017

Citation: Mazo Koné, Rachida Salah, Harir Noria. Human c-Cbl and Cbl-b Proteins are More Highly Expressed in the Thymus Compared to the Testis. Stem Cells Regen Med. 2017; 1(3): 1-3.

ABSTRACT

Background and Objectives: *c-Cbl and Cbl-b are two members of the Cbl family proteins, with a crucial role of down regulation of tyrosine kinase receptors. They act as E3 ubiquitin ligases and are multivalent adaptor proteins, making them important in maintaining homeostasis in the body. This study investigates the expression level in thymus and testis in normal conditions.*

Methods: *The expression level was assessed by immunochemistry of tissue microarrays of normal thymus and testis biopsies.*

Results: *Cbl-b and c-Cbl proteins were found to be highly expressed in normal testis and thymus, indicated as yellowish brown granules in the cytomembrane and cytoplasm compared to controls. The c-Cbl appears to be more highly expressed than the Cbl-b in the thymus, while c-Cbl appears slightly stronger than Cbl-b in the testis. The thymus was found with a higher grade compared to testis.*

Conclusion: *In this work we concluded, that in normal condition, thymus tissue expresses more Cbl family proteins (c-Cbl and Cbl-b) than the testis tissue in humans.*

Keywords

Human c-Cbl protein, Human Cbl-b protein, Testis, Thymus, Immunochemistry.

Introduction

The proto-oncogenic protein c-Cbl was discovered as the cellular form of v-Cbl, a retroviral transforming protein [1]. This was followed over the years by important discoveries, which identified c-Cbl and other Cbl-family proteins like Cbl-b as key players in several signalling pathways, like downregulation of tyrosine kinase receptors through an E3 ubiquitin ligase like process [2,3].

c-Cbl has donned the role of a multivalent adaptor protein, capable of interacting with a plethora of proteins, and has been shown to positively influence certain biological processes [4], making these proteins important in maintaining homeostasis in the body [4]. In the present study we examined the expression of c-Cbl and Cbl-b in normal human thymus and testis tissues using streptavidinperoxidase (SP) immuno-histochemical techniques to measure the expression profile and compare the grade in both thymus and testis.

Materials and Methods

Normal testis and thymus biopsies were collected from the Pathology Centre of the Hassani Abdel Kader (HAK) University College Hopital of Sidi Bel Abbes. The biopsy samples were fixed and transported in required condition at the Centre for anatomical-pathological examination. Tissues were fixed in 10% buffered-formalin and embedded in paraffin. Continuous sections of 4µm in thickness were sliced from paraffin blocks and immunostained for c-Cbl and Cbl-b.

The sections were suspended in a water bath, mounted on gelatinized glass slides, deparaffinised and soaked for 10 min at

room temperature in 3% H_2O_2 to block endogenous peroxidase. The mouse anti-human c-Cbl monoclonal antibody was produced by BD Biosciences and the mouse anti-human Cbl-b monoclonal antibody was produced by Santa Cruz (Santa Cruz Biotechnology, USA). These antibodies diluted at 1:100 were used as primary antibodies and were applied overnight at 4°C. The HRP (Horseradish peroxydase) detection reagent kit and the diaminobenzidin (DAB) (Daco Technology) solutions were used for staining detection according to the instruction manual. Our controls were normal testis and thymus slides with omission of the antibodies application step. The research was approved by the Pathology Centre that provides the biopsies in accordance with the University College Hospital Committee Board (UCHCB).

Results

Our results revealed that Cbl-b and c-Cbl proteins were highly expressed in normal testis and thymus normal compared to controls. The expression is indicated as yellowish brown granules in the cytomembrane and cytoplasm. c-Cbl appears to show a stronger yellowish brown staining compared to Cbl-b in the thymus, while c-Cbl appears slightly stronger than Cbl-b in the testis. The expression of both proteins is diffusely distributed, and the expression level in the thymus is higher than the testis for both Cbl-b and c-Cbl.

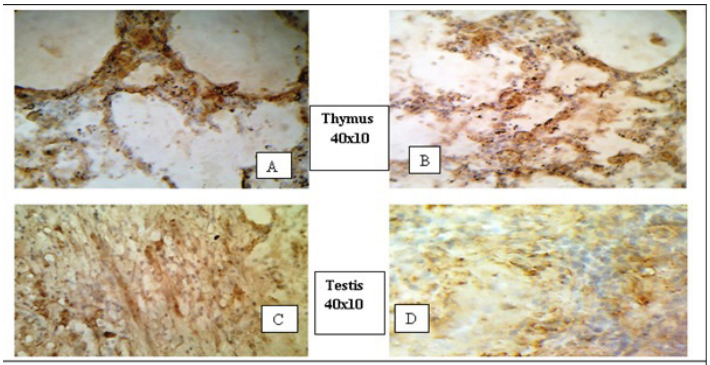

Figure: Cbl immunohistochemical staining:

A: c-Cbl diffuse expression with strong positive yellowish brown granules distributed in the cytoplasm or in the membrane of cells

B: Cbl-b diffuse expression with strong positive yellowish brown granules distributed in the cytoplasm or in the membrane of cells

C: Cbl-b diffuse expression with light positive yellowish brown granules distributed in the cytoplasm or in the membrane of cells

D: Cbl-b diffuse expression with light positive yellowish brown granules distributed in the cytoplasm or in the membrane of cells

Discussion

This study shows a higher grade of staining for Cbl proteins in the thymus compared to the testis. Our results are concordant with the results of Langdon et al., who found the thymus expression profile to be higher than the testis [6]. They did not use the technique of immunochemistry however; they conclude that Cbl is abundantly expressed in thymus and testis [7]. Taken individually, c-Cbl was higher than Cbl-b in the thymus and the same was observed in the testis, while both Cbl-b and c-Cbl were higher in thymus denoting their abundance in the thymus, a fact to support the role of these proteins in T-cell regulation [8,9].

The grades of Cbl expression were diffuse in over all cases showing that the cytoplasmic localization of the Cbl protein is consistent with previous studies [10]. A few studies on the expression level of Cbl proteins using the immunochemistry techniques are available and they address the expression in pathological tissues such as cancers. These studies find some correlation between the expression level and the poor prognostic outcomes in some cancers [4,10,11]. Our investigation clearly finds that Cbl family proteins are more highly expressed in the thymus than the testis from normal humans.

Conclusion

The literature indicates that Cbl family proteins are highly expressed in the testis and thymus from normal mice. In this work we concluded that human thymus tissue expresses more Cbl family proteins (c-Cbl and Cbl-b) than the human testis tissue in normal condition. However, because of human race complexity, a larger scale study with different races is needed to strengthen this investigation due to the possibility of variation between races.

Acknowledgement

This work was read and edited by Wallace Y. Langdon, PhD., School of Pathology and Laboratory Medecine, University of Western Australia-Australia. We thank especially Dr Harir Noria who supports the Research work by providing the antibodies for this research. We also thank the Pathology Centre and all the staff for their support in conducting this research.

References

1. Langdon WY, Hartley JW, Klinken SP, et al. v-cbl, an oncogene from a dual-recombinant murine retrovirus that induces early B-lineage lymphomas. Proc. Natl. Acad. Sci. U.S.A. 1989; 86:1168-1172.
2. Joazeiro CA, Wing SS, Huang H, et al. The tyrosine kinase negative regulator c-Cbl as a RING-type, E2-dependent ubiquitin-protein ligase. Science. 1999; 286: 309–312.
3. Levkowitz G, Waterman H, Ettenberg SA, et al. Ubiquitin ligase activity and tyrosine phosphorylation underlie suppression of growth factor signaling by c-Cbl/Sli-1. Molecular Cell. 1999; 4: 1029-1040.
4. Swaminathan G, Tsygankov AY. The Cbl family proteins: Ring leaders inregulation of cell signalling. Journal of Cellular Physiology. 2006; 209: 21-43.
5. Dong Q, Yun-Peng Liu, Xiu-Juan Qu, et al. Expression and clinical significance of c-Cbl, Cbl-b, and epidermal growth factor receptor in gastric carcinoma. Chinese Journal of Cancer. 2010; 29.
6. Langdon WY, Hyland CD, Grumont RJ, et al. The c-cbl proto oncogene is preferentially expressed in thymus and testis tissue and encodes a nuclear protein. Journal of Virology. 1989; 63: 5420-5424.
7. Thien C, Langdon WY. c-Cbl: A regulator of T cell receptor-mediated signalling. Immunology and cell biology. 1998; 76: 473-482.
8. Liu YC, Gu H. Cbl and Cbl-b in T-cell regulation. Trends in

Immunology. 2002; 23: 140-143.
9. Langdon WY. The cbl oncogene: a novel substrate of protein tyrosine kinases. Aust. N.Z.J. Med.1995; 25: 859-864.
10. Ito R, Nakayama H, Yoshida K, et al. 2004. Expression of Cbl linking with the epidermal growth factor receptor system is associated with tumor progression and poor prognosis of human gastric carcinoma. Virchows Arch. 2004; 444: 324-331.
11. Jiao X, Bo Jin, Xiujuan Qu, et al. Expressions of c-Cbl, Cbl-b and EGFR and Its Role of Prognosis in NSCLC. Chinese Journal of Lung Cancer. 2011; 14: 512-517.