Survivin expression in lymph nodes, affected by lymphoma and reactive hyperplasia

Mazur G[1], Hałoń A[2], Wróbel T[1], Urbaniak J[3], Kuliczkowski K[1], Woźniak M[3]

[1]Department of Haematology, Blood Neoplasms and Bone Marrow Transplantation,
[2]Department of Pathological Anatomy, [3]Department of Clinical Chemistry;
Wrocław Medical University, Poland

Abstract

Survivin is a member of the inhibitors from the apoptosis family over-expressed in various human cancers. The aim of the study was to evaluate the expression of survivin in lymph nodes, invaded by non-Hodgkin's lymphomas (nHL) and in lymph nodes reactive hyperplasia. We analysed paraffin sections obtained from 50 patients with nHL and from 13 patients with reactive hyperplasia using, an anti-survivin antibody. There was abundant immunoreactivity cytoplasm in 34/50 (68%) patients with nHL and in only 5/13 (38%) with reactive hyperplasia. Out of the 27 patients with aggressive nHL, 19 (70%) revealed survivin expression in almost all tumour cells. Out of the 23 patients with indolent nHL, 16 (70%) revealed the expression but with lower immunoreactivity score. The study indicates that patients with nHL presented a high level of survivin expression, it was more pronounced in aggressive than in indolent nHL.

Key words: survivin, lymph nodes, reactive hyperplasia, lymphomas.

Introduction

Survivin (also termed *Birc5*) is a protein that regulates cell division and inhibits apoptosis through a pathway, different from that, involving the bcl-2 family [1]. Survivin contains a baclovirus inhibitor of apoptosis repeat (BIR) protein domain, classifying it as a member of the inhibitor of the apoptosis protein (IAP) gene family [1, 2, 3]. Unique among other IAP proteins, is survivin highly expressed during embryonic and foetal development and in rapidly dividing cells, whereas it is undetectable in terminally differentiated normal adult tissues [3, 4, 5]. Its expression is regulated in a strict cell cycle-depended manner with the maximum level, occurring during the G2/M phase, and it localizes to mitotic spindle microtubules in a reaction, required for apoptosis inhibition [2, 4, 5]. Over expression of survivin has an oncogenic potential because it may overcome the G2/M phase checkpoint to enforce the progression of cells through mitosis [3]. Survivin suppresses apoptosis, induced by Fas, Bax, caspase-3, caspase-7 and anticancer drugs [3].

Survivin is abundantly expressed in the majority of cancers, including haematological malignancies [3, 5, 6, 7]. Survivin has been shown to be expressed in 60 cancer cell lines, including breast, lung, prostate, colon, pancreas, stomach, brain, melanoma, ovarian, renal cancer, as well as leukaemia/lymphoma [3, 4, 5, 8, 9].

Although either retrospective predictive or prognostic studies on the impact of the survivin pathway in solid tumour have been reported, little is still known about the potential role of this molecule in haematopoietic malignancies. In this study, we investigated the potential expression and localization of survivin in patients with non-Hodgkin's lymphomas and in patients with reactive lymph node hyperplasia as the control. We analyzed the relationship between its expression and malignant potential and the potential prognostic impact for disease progression and the clinical outcome of lymphoma.

Material and methods

The analysis was performed in patients with lymph node enlargement, diagnosed at the Department of Haematology, Blood Neoplasms and Bone Marrow Transplantation, Medical

ADDRESS FOR CORRESPONDENCE:
Grzegorz Mazur
Department of Haematology, Blood Neoplasms
and Bone Marrow Transplantation
Medical University of Wrocław
Pasteura 4 , 50-367 Wrocław, Poland
Phone: +48 71 7842599, Fax: +48 71 784 0112,
e-mail: grzegmaz@hemat.am.wroc.pl

Figure 1. A. Peripheral B-cell leukaemia/lymphoma; 210x. Survivin expression: grade I (<25%).

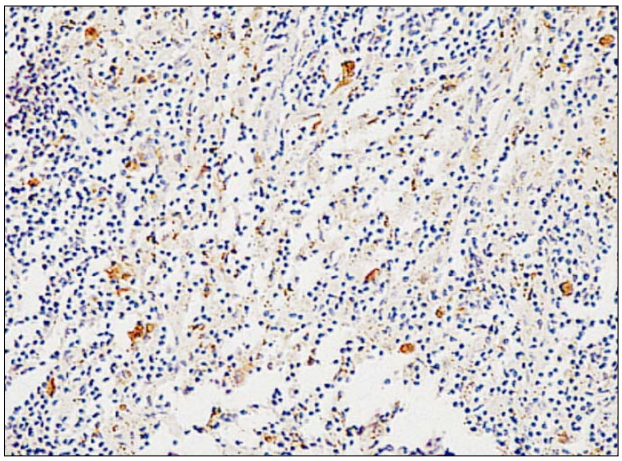
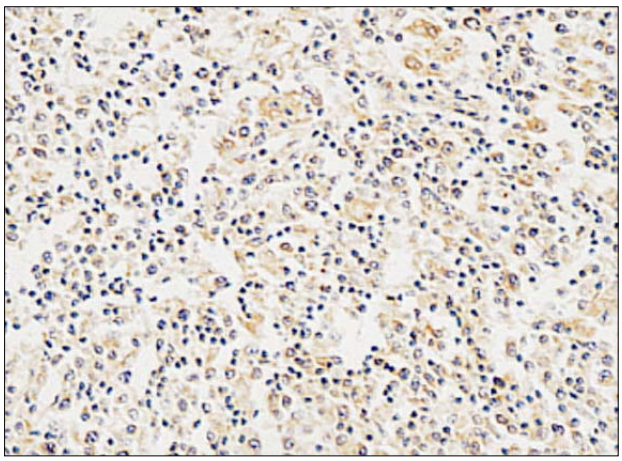


Figure 1. B. T-cell lymphoma; 210x. Survivin expression: grade IV (75-100%).



University in Wrocław. Samples were obtained from a total of 63 patients, including 50 patients with malignant non-Hodgkin's lymphoma (31 males, 19 females) and 13 patients with reactive lymph nodes hyperplasia (4 males, 9 females). The histological diagnosis of the enrolled patients with lymphomas was made, according to the REAL/WHO classification. Out of the 50 patients with lymphomas, 41 (82%) B-cell and 9 (18%) T-cell lymphomas were diagnosed. Aggressive lymphomas constituted a group of 27 cases (54%) and indolent ones - 23 (46%) cases. Histological classification of B-cell lymphomas revealed the following: peripheral leukaemia/lymphoma - 12 (29%), follicular lymphoma -7 (17%), mantle cell lymphoma - 6 (15%), immunoblastic/immunocytic - 5 (12%), other - 11 (27%). Immunohistochemical staining for survivin was performed, using an anti-survivin antibody (Chemicon International, USA) with formalin fixed, paraffin-embedded tissue samples. The investigated antigen was visualised, using the LSAB2 kit and diaminobenzidine (DAB). In each case, a control was included, in which the specific antibody was omitted. Evaluation of survivin expression based on immunoreactivity score of the percentage of positive cells was divided into four grades: I - <25% of positive cells; II - 26-50%; III - 51-75%; IV - 76-100%. Statistical analysis was performed, using the Fisher test. Differences were considered statistically significant at $p<0.05$.

Results

Survivin expression in the nucleus and/or cytoplasm was demonstrated in 62% of all the patients examined by immunohistochemistry (39 of 63). Scoring on the basis of the percentage of positive cells indicated survivin expression 34/50 (68%) patients with malignant lymphomas and in only 5/13 (38%) patients with lymph node reactive hyperplasia ($p<0.05$). Out of the 27 patients with aggressive lymphomas, 19 (70%) revealed survivin expression in virtually almost all tumour cells (grade III in 26% of cases, grade IV in 48% of cases). Out of the 23 patients with indolent lymphomas, 16 (70%) revealed survivin expression but with lower immunoreactivity score (grade I in 12, grade II in 4 cases).

Discussion

A considerable interest has recently focused around the critical role of apoptosis deregulation in the onset and progression of neoplasia and its ability to aberrantly prolong cell viability, facilitate the accumulation of mutations and promote drug resistance [6]. A candidate molecule to influence the cell death and cell viability balance in malignancies was identified in 1997: survivin, an apoptosis inhibitor of the IAP gene family [1, 2, 3, 4]. Present during foetal development, is survivin undetectable in terminally differentiated adult tissues [3, 5]. Its chromosomal location is 17q25, approximately 3% distance from the telomere and it comprises three introns and four exons, encoding 142 amino acids, including one copy of the baclovirus IAP repeat (BIR) essential for apoptosis inhibition [3, 5].

Survivin becomes prominently expressed in transformed cell lines and in all the most common human cancers of the colon, the stomach, the pancreas, the ovary, breast, the lung and prostate, in vivo [3, 5, 8, 9]. In most cancers, the expression of survivin correlated with reduced apoptotic index, poor prognosis and an increased risk of recurrence [2, 9]. Survivin over expression has been shown to be associated with aggressive forms of neuroblastoma and poor survival among patients with colorectal, stomach, breast, hepatocellular and non-small lung cancer [3, 4, 5, 8, 9]. Survivin expression was identified as an independent unfavourable prognostic factor in a randomized series of 69 acute myeloid leukaemia (AML) patients [6]. Although the expression of this protein did not seem to affect the complete remission (CR) rate, it significantly shortened the survival time of patients. Survivin is also found in approximately 50% of high-grade non-Hodgkin's lymphomas (centroblastic, immunoblastic), but not in low-grade lymphomas (lymphocytic) [2, 5]. Similarly, in our study, we demonstrated an increased expression of survivin not only in diffuse large B-cell lymphomas, but in other aggressive lymphomas, such as mantle cell lymphoma, follicular G3 lymphoma, lymphoblastic and Burkitt's lymphoma. Indolent lymphomas are characterized by a low proliferation index and impaired apoptosis. Interestingly enough, the expression of survivin in indolent lymphomas was much lower (predominantly grade I and II) than in aggressive lymphomas. This suggests that the inhibition of apoptosis in indolent lymphomas is related not only to survivin mechanisms. This observation requires further studies.

References

1. Chiou SK, Jones MK, Tarnawski AS. Survivin - an anti-apoptosis protein: its biological roles and implications for cancer and beyond. Med Sci Monit, 2003; 9: PI43-7.

2. Adida C, Haioun C, Gaulard P, Lapage E, Morel P, Briere J, Dombret H, Reyes F, Diebold J, Gisselbrecht C, Salles G, Altieri DC, Molina TJ. Prognostic significance of survivin in diffuse large B-cell lymphomas. Blood, 2000; 96: 1921-5.

3. Takai N, Miyazaki T, Nishida M, Nasu K, Miyakawa. Expression of survivin is associated with malignant potential in epithelial ovarian carcinoma. Int J Mol Med, 2002; 10: 211-6.

4. Verdecia MA, Huang H, Dutil E, Kaiser D, Hunter T, Noel JP. Structure of the human anti-apoptotic protein survivin reveals a dimeric arrangement. Nature, 2000; 7: 602-8.

5. Ambrosini G, Adida C, Altieri DC. A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. Nat Med, 1997; 8: 917-21.

6. Adida C, Recher C, Raffoux, Daniel MT, Taksin AL, Rousselot P, Sigaux F, Degos L, Altieri DC, Dombert H. Expression and prognostic significance of survivin in *de novo* acute myeloid leukaemia. Br J Haematol, 2000; 111: 196-203.

7. Siegel S, Wagner A, Schmitz N, Zeis M. Induction of antitumour immunity using survivin peptide-pulsed dendritic cells in a murine lymphoma model. Br J Haematol, 2003; 122: 911-4.

8. Casati C, Dalerba P, Rivoltini L, Galliano G, Deho P, Rini F, Belli F, Mezzanzanica D, Costa A, Andreola S, Leo E, Parmiani G, Castelli C. The apoptosis inhibitor protein survivin induces tumor-specific CD8+ and CD4+ T cells in colorectal cancer patients. Cancer Res, 2003; 63: 4507-15.

9. Tu SP, Jiang XH, Lin MCM, Cui JT, Yang Y, Lum CT, Zou B, Zhu YB, Jiang SH, Wong WM, Chan AO, Yuen MF, Lam SK, Kung HF, Wong BC. Suppression of survivin expression inhibits *in vivo* tumorigenicity and angiogenesis in gastric cancer. Cancer Res, 2003; 63: 7724-32.