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Expression of the cyclin-dependent kinase inhibitor p27 and its deregulation in mouse B cell lymphomas

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Abstract

CDKN1B (p27) regulates cell-cycle progression at the G1-S transition by suppressing the cyclin E/CDK2 kinase complex. In normal lymphocytes and most human B cell non-Hodgkin lymphomas (NHL), there is an inverse correlation between proliferative activity and expression of p27; however, a subset of NHL with high mitotic indices expresses p27, which is inactive due to sequestration in nuclear protein complexes or due to cytoplasmic retention. Our studies of mouse B cell NHL also identified cases with high proliferative activity and high levels of p27 at a surprisingly high frequency. Here, p27 was complexed with D-type cyclins 1 and 3 and with the COPS9 protein, JAB1. In addition, we found cytoplasmic sequestration following phosphorylation by activated AKT.

Keywords: p27; Mouse B cell lymphoma; Cell-cycle regulation

1. Introduction

Cell-cycle progression is controlled by the sequential activation of cyclin-dependent kinases (CDK) complexed with cyclins. CDK-bound cyclins activate CDK, promoting cell-cycle progression, while CDK inhibitors (CKI) negatively regulate the kinases and cause cell-cycle arrest [1]. CKI belong to two families: the INK4 family, comprising $p16^{INK4a}$, $p15^{INK4b}$, $p18^{INK4c}$, and $p19^{INK4d}$; and the CIP/KIP family of $p21^{CIP1}$, $p27^{KIP1}$, and $p57^{KIP2}$. Among these inhibitors, p27 regulates transition of the cell cycle from the G1 to S phase through its activity as a potent inhibitor of cyclin E or cyclin A complexed with CDK2 [1]. Because p27 must be localised to the nucleus to function as a CKI, mechanisms resulting in proteosomal degradation, nuclear export, or cytoplasmic retention will limit its activity and promote G1-S progression. In fact, the abundance of p27 is highly regulated at or after translation by a series of mechanisms. These include degradation mediated by the SCF ubiquitin protein ligase complex as well as proteolysis resulting in loss of the cyclin-binding domain [2–4]. At G1-S, p27 is phosphorylated by cyclin E/CDK2 on Thr187. The SCF complex recognises Thr187 and mediates polyubiquitination of the protein, thereby promoting its degradation via the proteosome. p27 degradation is also accelerated by JAB1 (COPS5), a protein that mediates nuclear export of p27 and is a positive regulator of the SCF complex [5]. Cytoplasmic sequestration of p27 results from phosphorylation on Ser10 by stathmin

Abbreviations: APCT, anaplastic plasmacytoma; B-CLL, B cell chronic lymphocytic leukæmia/lymphoma; BL, burkitt lymphoma; BLL, burkitt-like lymphoma; CBL, centroblastic DLBCL; CDK, cyclin-dependent kinase; CKI, CDK inhibitor; DLBCL, diffuse large B cell lymphoma; FBL, follicular B cell lymphoma; IBL, immunoblastic DLBCL; MALT, mucosal associated lymphoid tissue; MM, multiple myeloma; MZL, marginal zone lymphoma; NHL, non-Hodgkin lymphoma; SBL, small B cell lymphoma; SDS-PAGE, sodium dodecylsulfate polyacrylamide gel electrophoresis

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[6,7]. Finally, AKT phosphorylates Thr157, which acts as a docking site for 14-3-3, suppressing nuclear localisation [8–11].

Reduced levels of p27 are frequent in human breast and colon cancers and have been associated with poor prognosis [12–14]. The patterns of expression and role of p27 in development of human B cell lymphomas, however, are complex and perhaps less well understood. In general, there tends to be an inverse relationship between p27 expression and the growth fraction/proliferative index, as determined by Ki-67 protein expression, as well as the expression of cyclin E [15]. Low-growth-fraction lymphomas such as follicular, mantle cell, and B cell chronic lymphocytic leukæmia/lymphoma (B-CLL) are mostly p27-positive, while those with high growth fractions have low p27 levels [16,17]. This suggests that low-level p27 expression might be a prerequisite for sustained high-level proliferation, a finding supported by early studies [15]; however, it appears that normal growth regulation by p27 is undermined in a substantial number of lymphomas including some cases of both high-grade diffuse large B cell (DLBCL) and Burkitt lymphomas (BL) [18,19] and low-grade B-CLL [20].

Recently, others and we found that mice develop a wide spectrum of B cell-lineage lymphomas, some of which exhibit both histological and molecular similarities to human non-Hodgkin lymphoma (NHL) [21–25]. These studies resulted in the formulation of a consensus nomenclature that describes the classes of mouse neoplasms and their relationships to human B cell-lineage NHL [26]. Understanding these cross-species similarities and differences will be important for efforts to model human diseases in the mouse and to uncover aspects of normal B cell differentiation [27].

In this study, we have extended our analyses of these relationships by investigating the expression of p27 and its regulators in mouse B cell lymphomas of different histological types and differentiation stages. Here we describe the expression of p27 in relation to expression of Ki-67; cyclins D1, D3, A, and E; CDK2 and CDK4; SKP2, CUL1, and JAB1 for a large panel of primary mouse B cell tumours using a series of approaches including tissue arrays, quantitative PCR, and immunoblotting.

2. Materials and methods

2.1. Mice, tissue samples, and cell lines

Cell lines utilized in this study were cultured directly from primary lymphomas of NFS.V$^+$ congenic mice (NFS-216, small B cell lymphoma; NFS-215, NFS-227, Burkitt-like lymphomas) or λ-MYC transgenic mice (B6.SJ003-8, B6.SJ003-11, Burkitt lymphomas). We evaluated 237 B cell-lineage tumours (Supplemental Table 1), 4 normal spleens, and 10 spleens from mice with lymphoproliferative, nonmalignant changes as controls. Most lymphomas were from NFS.V$^+$ mice, strains congenic for ecotropic murine leukæmia proviruses of AKR or C58 mice that develop lymphomas at high incidence [23], and C57BL/6 mice bearing a λ-MYC transgene [28] (Supplemental Table 1). We also studied anaplastic and conventional plasmacytomas (APCT and PCT, respectively) that developed spontaneously in NFS.V$^+$ congenic mice [29]. The APCT exhibit histological features that suggest they are less mature than primary tumours of pristane-primed BALB/c mice [30]. Primary lymphomas were classified according to morphological, immunophenotypical, and molecular criteria outlined in the Bethesda classification of lymphoid neoplasms by a pathologist (TNF) who co-authored the classification [26]. We recognise three histologically and molecularly defined subsets of splenic marginal zone lymphoma (MZL) that comprise a progression from low-grade MZL to high-grade tumors—MZL+ and the most advanced MZL++ [22,29,31]. All of the B cell lymphomas had immunoglobulin heavy-chain gene rearrangements demonstrated by Southern blotting and/or B cell markers by immunophenotyping. Low-proliferation fraction lymphomas included small B cell lymphoma (SBL), follicular B cell lymphoma (FBL), and MZL. Lymphomas with a high proliferation index included Burkitt-like (BLL), Burkitt (BL), MZL+, MZL++, and DLBCL of centroblastic (CBL) and immunoblastic types (IBL). APCT were also found to have high proliferative fractions. The tissues were fixed in buffered formalin, embedded in paraffin for sectioning, and stained with hematoxylin and eosin (H&E).

2.2. Tissue microarray (TMA)

H&E slides from primary lymphomas were marked for areas with dominant populations of lymphoma cells that would be “cored” for generating a microarray containing multiple samples of each histological class of lymphoma on a single slide. A TMA was then manually constructed with an arrayer (Beecher Instruments Microarray Technology) using a 1-mm needle so that all samples selected could be arranged in a single block. Sections (4 μm) were cut from the TMA paraffin block and placed on Superfrost/Plus slides (Fisher Scientific). One section was stained with H&E and used for histological validation, and the others were used for immunostaining. The TMA H&E slide was read and the results compared with the previous H&E diagnosis. The pathologist responsible for the original diagnoses read the TMA H&E slide and the results were validated against the diagnoses made from the full section slides. Normal lymph node, bone marrow, and spleen from a healthy mouse were selected for controls.

Immunostaining of TMA was performed by the avidin-biotin peroxidase complex method using the Vectastain Elite ABC kit (Vector). Briefly, sections from paraffin blocks were heated at 60 °C for 1 h, deparaffinised, and rehydrated through xylene and alcohol. Endogenous peroxidase was blocked by incubation with 3% hydrogen peroxide in methanol for 5 min. After rinsing in tap water and phosphate-buffered saline (PBS), slides were heated in a pressure cooker