Review

Expression of p27^{Kip1} in Normal, Hyperplastic and Neoplastic Endocrine Tissues

Ricardo V. Lloyd

Department of Laboratory Medicine and Pathology, Mayo Clinic, 200 First Street SW, Rochester, MN 55905

Received for publication November 24, 1998

p27^{Kip1} (p27) is a member of the cyclin dependent kinase inhibitor (CDKI) family of cell cycle proteins. We analyzed p27 expression in normal endocrine cells, endocrine cell hyperplasia, adenomas and carcinomas. The levels of p27 protein expression decreased during tumor development and progression. This decrease occurs mainly

at the post-translational level with protein degradation by the ubiquitin-proteasome pathway. These results indicate that p27 may be a useful immunohistochemical marker to help separate normal from hyperplastic endocrine cells and adenomas from carcinomas.

Key words: Endocrine cells, p27, Cell cycle, Immunohistochemistry

I. Introduction

Studies over the past few years have shown that cyclins and cyclin-dependent kinase (CDK) complexes have important regulatory roles during cell cycle progression [10, 13, 19, 26]. Cyclin-CDK complexes are in turn regulated by the cyclin-dependent kinase inhibitors (CDKIs), that generally inhibit cell cycle progression.

The Cip/Kip family of CDKI includes p21/WAF1/CIP1 (p21), p27/kip1 (p27) and p57/kip2 (p27) [15, 20, 24]. These proteins inhibit kinase activities of pre-activated G1 cyclin E-CDK2, cyclin D-CDK4/6 and other cyclins. The Cip/Kip proteins are designated as universal CDKI because they interact with various CDK complexes, with cyclins A, E, D1, D2, D3 and CDKs [21]. Recent studies have shown that p27 can have prognostic and diagnostic significance in various human tumors [1, 2, 6–9, 12, 14, 16, 18, 21, 23, 25, 27–29, 31, 32].

We examined p27 expression in various endocrine tissues to determine if p27 expression can be used to distinguish between normal, hyperplastic and neoplastic endocrine tissue.

II. Materials and Methods

Immunohistochemical staining with an anti-p27 antibody used at a 1/1000 dilution (Transduction Laboratory, Lexington Kentucky) as previously reported [4, 5, 11, 16, 17].

The results of immunostaining were analyzed by counting 1000 cells per slide and the percentage of positive cells was expressed as a labeling index (LI).

III. Results

Normal tissues from thyroid, parathyroid and pituitary expressed higher levels of p27 compared to adenomas and carcinomas (Table 1). Immunostaining for p27 was intranuclear. Comparison of the normal rim of parathyroid adenoma to the adjacent adenomatous chief cells showed a marked increase in the stains of normal cells. Normal thyroid cells showed strong nuclear staining compared to weaker staining in the adjacent thyroid carcinoma.

Analysis of normal parathyroid and parathyroid hyperplasias showed lower levels of p27 expression in hyperplasia. Similarly parathyroid adenomas and carcinomas had lower levels of p27 protein compared to normal tissues (Table 1).

Correspondence to: R. V. Lloyd, M.D., Department of Laboratory Medicine and Pathology, Mayo Clinic, 200 First Street SW, Rochester, MN 55905.

Table 1. Expression of p27 in normal hyperplastic and neoplastic endocrine tissues.

Tissue/Diagnosis	p27 Labeling Index
Thyroid	
Normal	74±4.9
Follicular adenoma	48±5.6 *
Follicular carcinoma	16±2 ***
Papillary carcinoma	12±3.0 ***
Anaplastic carcinoma	9.4±1.7 ***
Parathyroid	
Normal	90±1.4
Hyperplasia	68±2.5 *
Adenoma	57±3.4 **
Carcinoma	14±2.6 ***
Pituitary	
Normal	79±4
Adenoma	64±9 *
Carcinoma	44±7.5 *

* p<0.05; ** p<0.01; *** p<0.001 compared to normal tissue.

IV. Discussion

We have previously shown that p27 is frequently expressed in endocrine cells as well as in other cell types. Our studies have also shown decreased expression of p27 in thyroid adenomas and carcinomas compared to normal thyroid. Interestingly, the decreased levels of p27 in papillary and anaplastic carcinomas are at similar levels, in spite of the more aggressive behavior of anaplastic carcinoma. These observations indicate that a) p27 is not a sensitive enough marker to distinguish between different types of thyroid carcinomas and b) other CDKIs in addition to p27 are probably changed during thyroid tumor progression.

Analysis of p27 protein expression in endocrine tumor was first reported by Lloyd *et al.* [16]. They observed decreased expression of p27 in endocrine adenomas and carcinomas compared to normal tissues. However, the changes are not as striking as identified in some non-endocrine tissues such as breast, prostate and colonic carcinomas. For example, in a study of thyroid carcinomas, Erickson *et al.* [4] found similar levels of p27 in high- and low-grade cancers, i.e., anaplastic carcinomas and papillary carcinomas. Similar findings were observed in a comparison of different types of papillary carcinomas by others [5, 17]. In a large series of parathyroid tumors, there was significantly decreased p27 expression in carcinomas compared to adenomas. Jin *et al.* [11] reported that in pituitary tumors, the p27 protein levels decreased during progression from adenomas to carcinomas, but the differences were moderate compared to other non-endocrine tumors. Jin *et al.* [11] showed that regulation in the pituitary was post-translational since the mRNA levels were similar in normal and tumorous pituitaries. Similar findings were reported in parathyroid tumors by Erickson

et al. [5]. In ACTH secreting pituitary tumors, Dahia *et al.* [3] reported that corticotroph adenomas express p27 protein, while one carcinoma in their series showed loss of p27 protein expression. Analysis of three other pituitary carcinomas by the same investigators showed two tumors with loss of expression, while one case showed moderate expression.

The relationship of decreased levels of p27 protein and tumor progression is variable in different endocrine tumors. Our studies of adrenal cortical and medullary tumors showed only slight differences between normal benign and malignant tumors with respect to p27 protein expression [16]. Yatabe *et al.* [14] also reported a higher level of p27 in small cell neuroendocrine lung carcinomas compared to non-small cell tumors. Since small cell lung carcinomas have a high proliferation rate, one would predict low levels of p27 protein, since in many tissues there is an inverse relationship between tumor proliferation and p27 expression. However, small cell carcinoma is known to have genetic defects such as in the Rb and p53 tumor suppressor genes and over expression of c-myc [22] that might allow this neoplasm to proliferate in spite of high p27 levels. Recent reports suggest that c-myc could overcome p27-induced growth arrest by allowing cyclin E-CDK2 to function in the presence of elevated levels of p27 [30].

In summary, the CDKI p27 can be used as a diagnostic marker in some endocrine tissues and as a prognostic marker in some tumors.

V. References

1. Cheville, J. C., Lloyd, R. V., Sebo, T. J., Cheng, L., Erickson, L., Bostwick, D. G., Lohse, C. M. and Wollan, P.: Expression of p27^{kip1} in prostatic adenocarcinoma. *Mod. Pathol.* 11; 324-328, 1998.
2. Ciaparrone, M., Yamamoto, H., Yao, Y., Sgambato, A., Cattoretti, G., Tomita, N., Monden, T., Rotterdam, H. and Weinstein, I. B.: Localization and expression of p27^{kip1} in multistage colorectal carcinogenesis. *Cancer Res.* 58; 114-122, 1998.
3. Dahia, P. L., Aguiar, R. C., Honegger, J., Fahlbush, R., Jordan, S., Lowe, D. G., Lu, X., Clayton, R. N., Besser, G. M. and Grossman, A. B.: Mutation and expression analysis of the p27^{kip1} gene in corticotropin-secreting tumors. *Oncogene* 16; 69-76, 1998.
4. Erickson, L. A., Jin, L., Wollan, P. C., Thompson, G. B., van Heerden, J. and Lloyd, R. V.: Expression of p27^{kip1} and Ki-67 in benign and malignant thyroid tumors. *Mod. Pathol.* 11; 169-174, 1998.
5. Erickson, L. A., Jin, L., Wollan, P., Thompson, G. B., van Heerden, J. A. and Lloyd, R. V.: Parathyroid hyperplasia, adenomas and carcinomas: differential expression of p27^{kip1} protein. *Am. J. Surg. Pathol.*, 1998 (in press).
6. Esposito, V., Baldi, A., DeLuca, A., Groger, A. M., Loda, M., Giordano, G. G., Caputi, M., Baldi, F., Pagano, M. and Giordano, A.: Prognostic role of the cyclin-dependent kinase inhibitor p27 in non-small cell lung cancer. *Cancer Res.* 57; 3381-3385, 1997.
7. Feldman, R. M. R., Correll, C. C., Kaplan, K. B. and Deshaies, R. J.: A complex of Cdc4p, Skp1p and Cdc53p/Cullin cata-

- lyzes ubiquitination of the phosphorylated CDK inhibitor Sic1p. *Cell* 91; 221–230, 1997.
8. Florenes, V. A., Maelandsmo, G. M., Kerbel, R. S., Slingerland, J. M., Nesland, J. M. and Holm, R.: Protein expression of the cell-cycle inhibitor p27^{Kip1} in malignant melanoma: Inverse correlation with disease-free survival. *Am. J. Pathol.* 153; 305–312, 1998.
 9. Fredersdorf, S., Burns, J., Milne, A. M., Packham, G., Fallis, L., Gillett, C. E., Royds, J. A., Peston, D., Hall, P. A., Hanby, A. M., Barnes, D. M., Shousha, S., O'Hare, M. J. and Lu, X.: High level expression of p27^{Kip1} and cyclin D1 in some human breast cancer cells: inverse correlation between the expression of p27^{Kip1} and degree of malignancy in human breast and colorectal cancers. *Proc. Natl. Acad. Sci. USA* 94; 6380–6385, 1997.
 10. Hunter, T. and Pines, J.: Cyclins and cancer. II. Cyclin D and CDK inhibitors come of age. *Cell* 79; 573–582, 1994.
 11. Jin, L., Qian, X., Kulig, E., Sanno, N., Scheithauer, B. W., Kovacs, K., Young, W. F. Jr. and Lloyd, R. V.: Transforming growth factor-beta, transforming growth factor-beta receptor II and p27^{Kip1} expression in nontumorous and neoplastic human pituitaries. *Am. J. Pathol.* 151; 509–519, 1997.
 12. Jordan, R. C., Bradley, G. and Slingerland, J.: Reduced levels of the cell-cycle inhibitor p27^{Kip1} in epithelial dysplasia and carcinoma of the oral cavity. *Am. J. Pathol.* 152; 585–590, 1998.
 13. Kamb, A.: Cell-cycle regulators and cancer. *Trends Genet.* 11; 136–140, 1995.
 14. Kawana, H., Tamaru, J., Tanaka, T., Hirai, A., Saito, Y., Kitagawa, M., Mikata, A., Harigaya, K. and Kuriyama, T.: Role of p27^{Kip1} and cyclin-dependent kinase 2 in the proliferation of non-small cell lung cancer. *Am. J. Pathol.* 153; 505–513, 1998.
 15. Lee, M. H., Reynisdottir, L. and Massague, J.: Cloning of p57 Kip2, a cyclin-dependent kinase inhibitor with unique domain structure and tissue distribution. *Genes Dev.* 9; 639–649, 1995.
 16. Lloyd, R. V., Jin, L., Qian, X. and Kulig, E.: Aberrant p27^{Kip1} expression in endocrine and other tumors. *Am. J. Pathol.* 150; 401–407, 1997.
 17. Lloyd, R. V., Ferrero, J. A., Jin, L. and Sebo, T.: TGF β , TGF β receptors, Ki-67 and p27^{Kip1} expression in papillary thyroid carcinomas. *Endocr. Pathol.* 4; 293–300, 1997.
 18. Loda, M., Cukor, B., Tam, S. W., Lavin, P., Fiorentino, M., Draetta, G. F., Jessup, J. M. and Pagano, M.: Increased proteasome-dependent degradation of the cyclin-dependent kinase inhibitor p27 in aggressive colorectal carcinoma. *Nature Med.* 3; 231–234, 1997.
 19. Massague, J. and Polyak, K.: Mammalian antiproliferative signals and their targets. *Curr. Opin. Genet. Dev.* 5; 91–96, 1995.
 20. Matsuoka, S., Edwards, M. C., Bai, C., Parker, S., Zhang, P., Baldini, A., Harper, J. W. and Elledge, S. J.: p57/Kip2, a structurally distinct member of the p21/CIP1 Cdk inhibitor family, is a candidate tumor suppressor gene. *Genes Dev.* 9; 650–662, 1995.
 21. Millard, S. S., Yan, J. S., Nguyen, H., Pagano, M., Kiyokawa, H. and Koff, A.: Enhanced ribosomal association of p27^{Kip1} mRNA is a mechanism contributing to accumulation during growth arrest. *J. Biol. Chem.* 272; 7093–7098, 1997.
 22. Minna, J. B., Nau, M. M., Chiba, I. and Ihde, D. C.: Lung cancer. In "Principles and Practice of Oncology", ed. by V. T. Denita, S. Rosenberg and S. Hellmen, JB Lippincott Philadelphia, 1989, pp. 591–594.
 23. Mori, M., Mimori, K., Shiraishi, T., Tanaka, S., Ueo, H., Susimachi, K. and Akiyoshi, T.: p27 expression and gastric carcinoma. *Nature Med.* 3; 593, 1997.
 24. Polyak, K., Lee, M. H., Erdjument-Bromage, H., Koff, A., Roberts, J. M., Tempst, P. and Massague, J.: Cloning of p27^{Kip1}, a cyclin-dependent kinase inhibitor and a potential mediator of extracellular antimitogenic signals. *Cell* 78; 59–66, 1994.
 25. Porter, P. L., Malone, K. E., Heagerty, P. J., Alexander, G. M., Gatti, L. A., Firpo, E. J., Daling, J. R. and Roberts, J. M.: Expression of cell-cycle regulators p27^{Kip1} and cyclin E, alone and in combination, correlate with survival in young breast cancer patients. *Nature Med.* 3; 222–225, 1997.
 26. Sherr, C. J. and Roberts, J. M.: Inhibitors of mammalian G1 cyclin-dependent kinases. *Genes Dev.* 9; 1149–1163, 1995.
 27. Singh, S. P., Lipman, J., Goldman, H., Ellis, F. H. Jr., Aizenman, L., Cangi, M. G., Signoretti, S., Chiaur, D. S., Pagano, M. and Loda, M.: Loss or altered subcellular localization of p27 in Barrett's associated adenocarcinoma. *Cancer Res.* 58; 1730–1735, 1998.
 28. Tan, P., Cady, B., Wanner, M., Worland, P., Cukor, B., Magi-Galluzzi, C., Lavin, P., Draetta, G., Pagano, M. and Loda, M.: The cell cycle inhibitor p27 is an independent prognostic marker in small (T_{1a,b}) invasive breast carcinoma. *Cancer Res.* 57; 1259–1263, 1997.
 29. Tshilias, J., Kapusta, L. R., DeBuer, G., Morava-Protzner, I., Zbieranowski, I., Bhattacharya, N., Catzavelos, G. C., Klotz, L. H. and Slingerland, J. M.: Loss of cyclin-dependent kinase inhibitor p27^{Kip1} is a novel prognostic factor in localized human prostate adenocarcinoma. *Cancer Res.* 58; 542–548, 1998.
 30. Vlach, J., Hennecke, S., Alevizopoulos, K., Conti, D. and Amati, B.: Growth arrest by the cyclin-dependent kinase inhibitor p27^{Kip1} is abrogated by c-Myc. *EMBO J.* 15; 6595–6604, 1996.
 31. Yang, R. M., Naitoh, H. J., Murphy, M., Wang, H. J., Phillipson, J., DeKernion, J. B., Loda, M. and Reiter, R. E.: Low p27 expression predicts poor disease-free survival in patients with prostate cancer. *J. Urol.* 159; 941–945, 1998.
 32. Yatabe, Y., Masuda, A., Koshikawa, T., Nakamura, S., Kuroishi, T., Osada, H., Takahashi, T., Mitsudomi, T. and Takahashi, T.: p27^{Kip1} in human lung cancers: differential changes in small cell and non-small cell carcinomas. *Cancer Res.* 58; 1042–1047, 1998.