Tumor protein D54: A promising marker of mucoepidermoid carcinoma

Atsutoshi Yaso*, Takaaki Kamatani, Yoshiki Mukudai, Yuzo Abe and Tatsuo Shirota

Received: 11 June 2021 / Accepted: 10 August 2021

Abstract
A definitive diagnosis of salivary gland tumors is extremely difficult to make without evaluating the entire tumor and conducting immunohistochemical examinations. In this study, we aimed to examine and compare the expression patterns of the tumor protein (TP) D52 family, including TPD52, TPD53, and TPD54, in salivary gland tumor cells by using immunohistochemical staining. Among over 30 benign and malignant salivary gland tumors with extensive and diverse morphological features and overlapping histological similarities, we selected Warthin’s tumor and pleomorphic adenoma to represent benign salivary gland tumors and mucoepidermoid carcinoma to represent malignant ones. Tumor samples were fixed in 10% buffered formalin and embedded in paraffin. Then, immunohistochemical staining was performed using antibodies against TPD52, TPD53, and TPD54. Neither the benign salivary gland tumors nor mucoepidermoid carcinoma stained for TPD52. However, the intensity of TPD53 and TPD54 staining was found to be low in the benign salivary gland tumors and high in mucoepidermoid carcinoma. TPD54 may serve as a pathological indicator of benign salivary gland tumors and mucoepidermoid carcinoma.

Key words: tumor protein D52 family, TPD54, mucoepidermoid carcinoma, immunohistochemistry

Introduction
The salivary glands are exocrine organs that produce saliva and are complex tissues composed of ductal, acinar, myoepithelial, and basal cells. Collectively called as luminal cells, ductal and acinar cells are present on the luminal side of the salivary duct system. Myoepithelial and basal cells are located on the basement membrane around the luminal cells and are thus called abluminal cells. In general, 3 types of acini (namely serous, mucinous, and mixed) and ducts (i.e., intercalated, striated, and excretory) are found in the salivary glands. The acini and intercalated ducts are surrounded by myoepithelial cells, whereas the striated and excretory ducts are surrounded by basal cells.

Tumors of the salivary glands comprise less than 1% of all neoplasms in the body; however, there are more than 30 benign and malignant salivary gland tumors with extensive and diverse morphologies yet overlapping histological similarities. Hence, it is extremely difficult to definitively diagnose salivary gland tumors without evaluating the entire tumor and conducting immunohistochemical examinations.

Expression patterns of the tumor protein (TP) D52 family, including TPD52, TPD53, TPD54, and TPD55, have been investigated in malignant tumors. TPD52, also known as CRHSP-28m, was first identified as a chromosome 8q21 amplification target in breast cancer and was subsequently detected in lung, prostate, ovarian, endometrial, and hepatic cancers. TPD53 has been identified as a novel 14-3-3-binding partner in breast cancer, with the highest level of expression observed at the G2-M transition; dysregulated expression of TPD53 results in incomplete mitosis. TPD54 has been shown to affect the proliferation, adhesion, and invasion of oral cancer cells; regulate the expression of pyruvate dehydrogenase; and

* Corresponding author
✉ Atsutoshi Yaso
yaso_80@dent.showa-u.ac.jp

Department of Oral and Maxillofacial Surgery, Division of Maxillofacial Surgery, School of Dentistry, Showa University, 2-1-1 Kitasenzoku, Ota-ku, Tokyo 145-8515, Japan.
influence the sensitivity of breast cancer cells to metformin. All members of the TPD52 family contain a coiled-coil structural motif that mediates homomeric and heteromeric interactions among them. We have previously evaluated the role of the TPD52 family in chondrocytes and oral squamous cell carcinoma (OSCC) cells. In OSCC cells, TPD54 is highly expressed both in the cancerous tissue and in the surrounding connective tissue, regardless of the tumor differentiation level. However, the expression of TPD52 in both highly and poorly differentiated OSCC cells is lower than that of TPD54; besides, TPD52 is barely expressed in normal tissue, whereas TPD53 is moderately expressed in cancer tissue. TPD54 might act as a negative regulator of tumor progression. Although the TPD52 family has been investigated in many cancers, their expression and function in salivary gland tumors still remain unclear. Several efforts have been made to identify new prognostic and diagnostic molecular markers and chemotherapeutic targets for salivary gland tumors.

While calponin is believed to be a useful and highly specific marker for identifying myoepithelial cells, all luminal and abluminal cells of the salivary glands stain positively for pan-cytokeratin. Nevertheless, there is no reliable immunohistochemical marker to distinguish benign salivary gland tumors from mucoepidermoid carcinoma. Here, we hypothesized that the immunohistochemical examination of the expression of the TPD52 family could be useful in the pathological diagnosis of salivary gland tumors.

Materials and methods

1. Tissue samples

The present study was approved by the Institutional Ethics Committee of Showa University Hospital ( Permit Number: 2016-002). We used pleomorphic adenoma of the parotid gland (5 cases) and Warthin’s tumor of the parotid gland (5 cases) as representatives of benign salivary gland tumors and mucoepidermoid carcinoma of the parotid (2 cases) and minor salivary glands (8 cases) as a representative of malignant salivary gland tumors. Cancer-free submandibular gland tissue excised during radical neck dissection for OSCC (5 cases) and minor salivary glands of the lip (5 cases) were selected as normal salivary gland tissue specimens.

2. Immunohistochemical analysis of paraffin-embedded tissue sections

The specimens were fixed in 10% buffered formalin and then embedded in paraffin wax. Serial sections (4 µm thick) collected from the tissue blocks were placed on silane-coated glass slides. Antigen retrieval was carried out by incubation with citrate-phosphate buffer (0.01 M, pH 6.0) at 121°C for 20 min. Endogenous peroxidases were blocked by incubating the samples with 10% hydrogen peroxide for 10 min. The sections were incubated at 4°C overnight with the following primary rabbit polyclonal antibodies: anti-TPD52 antibody (1:50; orbit 100564; Biornbyt, Cambridge, UK), anti-TPD53 antibody (1:200; 14732-1-AP; Proteintech, Rosemont, IL USA), and anti-TPD54 antibody (1:200; 11795-1-AP; Proteintech, Rosemont, IL USA). Anti-calponin 1 (1:100, rabbit monoclonal; ab46794; Abcam, Cambridge, UK) and anti-pan-cytokeratin (1:250, mouse monoclonal; ab7753; Abcam, Cambridge, UK) antibodies were also used for the immunohistochemical staining of calponin and pan-cytokeratin, respectively. The sections were incubated with secondary antibodies (EnVision + system-HRP labeled polymer anti-rabbit / anti-mouse; Dako, Glostrup, Denmark) prior to color development with a 3,3′-diaminobenzidine peroxidase substrate kit (Dako, Glostrup, Denmark) and were subsequently counterstained with hematoxylin. Immunohistochemical processes were conducted manually without using a slide preparation system. All images were captured using the Olympus BX51 microscope and Olympus CellSens Standard software (Olympus Corporation, Tokyo, Japan). Each immunohistochemical specimen was evaluated by 2 independent dentists and quantitatively scored based on the staining intensity (i.e., 0, no staining; 1, weak staining; 2, moderate staining; and 3, strong staining). The samples were evaluated twice at different times.

3. Statistical analysis

The scores of immunohistochemical staining for TPD52, TPD53, and TPD54 were compared by one-way analysis of variance. The Tukey-Kramer method was used to determine which means among a specific group of means were statistically different. All calculations were performed using the R package, with the level of statistical significance set at P<0.01. Statistically significant results are marked by an asterisk (*) in graphs.

Results

We used a squamous cell carcinoma specimen as the positive control (Fig. 1), as reported previously.

SUJMS 34.1–10, March 2022

Atsutoshi Yaso, et al.: Tumor protein D54: Mucoepidermoid carcinoma’s marker
All the samples were collected during salivary gland tumor resection (Table 1).

1. **Normal salivary glands**

When evaluating the normal salivary gland tissue (Fig. 2A), we observed high-intensity staining for calponin in myoepithelial cells at the periphery of the acini, intercalated ducts, and striated ducts (Fig. 2B); moderate-intensity staining for pan-cytokeratin in ductal cells (Fig. 2C); negative staining for TPD52 in acinar and ductal cells (Figs. 2D & 2G); high- and moderate-intensity staining for TPD53 in mucinous and serous acini, respectively (Figs. 2E & 2H); and moderate-intensity staining for TPD54 in mucinous acini and ductal cells (Figs. 2F & 2I).

2. **Pleomorphic adenoma**

In the case of pleomorphic adenoma (Fig. 3A), myoepithelial cells showed diffuse, moderate-intensity staining for calponin (Fig. 3B). Duct-like cells displayed diffuse, high-intensity staining for pan-cytokeratin (Fig. 3C). Pleomorphic adenoma cells were negative for TPD52 staining (Figs. 3D & 3G). Mucinous acini and ductal cells—positively stained for calponin—were moderately stained for TPD53 (Figs. 3E & 3H). TPD54 staining intensity was moderate and diffuse in both ductal cells and interstitial cellular components, including epithelioid and spindle-shaped cells (Figs. 3F & 3I).

3. **Warthin’s tumor**

Warthin’s tumors (Fig. 4A) did not stain for calponin (Fig. 4B), whereas high-intensity pan-cytokeratin staining was observed in the epithelial bilayer—consisting of a layer of tall, columnar, luminal cells encompassed by cuboidal oncotypic cells (Fig. 4C). Warthin’s tumors exhibited negative staining for TPD52 (Figs. 4D & 4G). The intensity of staining for TPD53 (Figs. 4E & 4H) and TPD54 (Figs. 4F & 4I) was found to be moderate in oncotypic cells on the outer side of the double layer of epithelial cells resting on the dense lymphoid stroma.

4. **Mucoepidermoid carcinoma**

According to the Armed Forces Institute of Pathology (AFIP) grading system, mucoepidermoid carcinoma is classified into 3 types: low, intermediate, and high-grade (Table 2). Immunohistochemical analysis of mucoepidermoid carcinoma (Fig. 5A) revealed extremely low-intensity staining for calponin in squamous cells (Fig. 5B) as well as low-intensity pan-cytokeratin...
Fig. 2. Expression of the TP52 family in normal salivary gland tissue
(A) Hematoxylin and eosin staining. (B) High-intensity calponin staining in myoepithelial cells at the periphery of the acini, intercalated ducts, and striated ducts. (C) Ductal cells showing moderate-intensity pan-cytokeratin staining. (D) and (G) Negative staining for TP52. (E) and (H) High-intensity TP53 staining in acini cells and low-intensity TP53 staining in ductal cells. (F) and (I) Moderate-intensity staining for TP54 in acini and ductal cells.
TP, tumor protein.

Fig. 3. Expression of the TP52 family in pleomorphic adenoma
(A) Hematoxylin and Eosin staining. (B) Diffuse, moderate-intensity calponin staining in myoepithelial cells and periphery cell nests. (C) Diffuse, high-intensity pan-cytokeratin staining in duct-like cells. (D) and (G) Negative staining for TP52 in pleomorphic adenoma. (E) and (H) Mucinous acini and intercalated duct cells stained moderately for TP53. (F) and (I) Diffuse, moderate-intensity TP54 staining in both ductal cells and interstitial cellular components consisting of epithelioid and spindle-shaped cells.
staining in mucus-secreting and squamous cells (Fig. 5C). Mucoepidermoid carcinoma cells did not stain for TPD52 (Figs. 5D & 5G). High-intensity staining for TPD53 (Figs. 5E & 5H) and TPD54 (Figs. 5F & 5I) was observed in mucous cells. The immunohistochemical staining scores for each specimen are summarized in Table 3. The signal intensity of TPD53 and TPD54 did not have any significant relationship with the histological grades of mucoepidermoid carcinoma.

Table 2. Histological evaluation of mucoepidermoid carcinoma

| Case No. | Intracytic component | Neural invasion present | Necrosis present | Mitosis (4 or more per 10 HPF*) | Anaplasia present | Grade   |
|----------|----------------------|-------------------------|-----------------|--------------------------------|-----------------|---------|
| 1        | 2                    | 2                       | 0               | 0                              | 0               | Low     |
| 2        | 0                    | 0                       | 0               | 0                              | 0               | Low     |
| 3        | 0                    | 0                       | 0               | 0                              | 4               | Low     |
| 4        | 2                    | 0                       | 0               | 0                              | 4               | Intermediate |
| 5        | 0                    | 0                       | 0               | 3                              | 4               | High    |
| 6        | 2                    | 0                       | 0               | 3                              | 4               | High    |
| 7        | 2                    | 0                       | 0               | 0                              | 4               | Intermediate |
| 8        | 0                    | 0                       | 3               | 0                              | 0               | Low     |
| 9        | 2                    | 0                       | 0               | 0                              | 0               | Low     |
| 10       | 0                    | 0                       | 0               | 0                              | 0               | Low     |

HPF, high-power field.
Comparison of immunohistochemical staining scores

The mean score of TPD52 staining was $0.5 \pm 0.53$ for normal tissue, $0.9 \pm 0.88$ for benign salivary gland tumors, and $0.6 \pm 0.52$ for mucoepidermoid carcinoma. The mean score of TPD53 staining was $2.7 \pm 0.48$ for normal tissue, $2.2 \pm 0.63$ for benign salivary gland tumors, and $2.4 \pm 1.16$ for mucoepidermoid carcinoma. The mean score of TPD54 staining was $1.3 \pm 0.68$ for normal tissue, $1.9 \pm 0.32$ for benign salivary gland tumors, and $2.5 \pm 0.53$ for mucoepidermoid carcinoma. Only the TPD54 staining score of mucoepidermoid carcinoma differed significantly from that of the normal salivary gland tissue ($P<0.01$) (Fig. 6).

Discussion

Salivary gland tumors display significant morphological diversity. Pleomorphic adenoma is the most common benign salivary gland tumor that consists of epithelial and mesenchymal cell components with wide variety in morphology. On the other hand, mucoepidermoid carcinoma is the most frequently occurring malignant salivary gland tumor involving mucus-secreting and squamous cells. The differential diagnosis of mucoepidermoid carcinoma is broad and depends on tumor grade and morphology (e.g., the presence of oncocyes or clear cells). Warthin’s tumor, also known as adenolymphoma or cystic papillary adenoma, is a benign salivary gland tumor that involves the lymphoid stroma and glandular epithelium with a characteristic eosinophilic cytoplasm. Neoplastic myoepithelial cells show diverse patterns in salivary gland tumors. Therefore, there is a need to identify novel biomarkers for the differential diagnosis of salivary gland tumors.

TPD52 is a member of a small protein family including TPD52, TPD53, TPD54, and TPD55, which were identified in 1995, 1996, 1998, and 2006, respectively. TPD52 is normally overexpressed in exocrine cells that contain large secretory granules. Furthermore, it regulates exocytotic secretion and vesicle trafficking, which may play an important role in the calcium-dependent membrane trafficking necessary for cytokinesis in rapidly proliferating malignant cells. Animal studies have shown that TPD52 is expressed along with early endosomal markers in rat pancreatic acinar cells. TPD52 is predominantly localized in the cytoplasm of ovarian carcinoma cells and is frequently observed in mucinous and clear cell carcinomas. The
gene encoding TPD52 is located at chromosome 8q21.13 (i.e., a frequently amplified region in breast cancer), which is related to cell survival, proliferation, migration, and invasion. Clinically, TPD52 is associated with a poor prognosis in breast cancer patients. It has also been reported that TPD52 expression is influenced by posttranscriptional modifications that independently affect messenger ribonucleic acid stability. TPD53 is implicated in membrane trafficking because of its ability to bind to 14-3-3 proteins—a family of multifunctional, cytoplasmic molecules that negatively regulate the G2-M transition during mitosis. TPD53 is a cell cycle regulator that is highly expressed during the G2 to M transition. TPD54 is found on numerous small vesicles throughout the cell, functioning as a membrane trafficking protein. It not only affects cell proliferation, adhesion, and invasion, but also serves as a negative regulator of cell growth in breast cancer and OSCC. In addition, TPD54 inhibits anchorage-independent growth and cell migration in vitro and attenuates tumor growth in vivo; therefore, TPD54 may act as a negative regulator of tumor progression in OSCC cells. The expression of TPD55 is restricted to the testes, indicating a role in testis development and spermatogenesis. Hence,
we did not include TPD55 in this study. Based on the distribution of the TPD52 family members in human cells, we selected TPD52, TPD53, and TPD54 for immunohistochemical evaluation in salivary gland tumors.

TPD54 expression may be a novel prognostic marker for cancer, because TPD54 has been shown to be upregulated in several types of solid malignant tumors. Moreover, it has been demonstrated that the knockdown of TPD54 in OSCC cell lines inhibits cell growth, promotes cell apoptosis, and inhibits extracellular matrix-dependent cell migration and attachment in OSCC patients. Here, we observed that TPD52 was expressed neither in normal nor in tumorous salivary gland tissue. The salivary glands are involved in exocytotic secretion and cell vesicle trafficking; however, TPD52 may not contribute to these roles.

To the best of our knowledge, this is the first study to demonstrate the expression of TPD54 in mucoepidermoid carcinoma. TPD54 is upregulated in several types of solid malignancies and is a tumor protein responsible for the high proliferation rate of cancer cells. The expression of TPD54 has been reported to be significantly upregulated in prostate cancer tissue compared to the adjacent normal prostate tissue, indicating its critical role in the progression of prostate cancer. In this study, however, there was no relation between the expression of TPD54 and the grade of mucoepidermoid carcinoma. Thus, to elucidate the role of the TPD52 family in salivary gland tumors, future studies should follow up disease recurrence and metastasis for a substantial period of time. Moreover, knockdown and overexpression analyses in salivary gland tumor cell lines are required to understand the biological function of the TPD52 family in salivary gland tumors.

There was no relationship between the AFIP grades, assigned using the AFIP grading system recommended by the World Health Organization, and the intensity of staining for the TPD52 family. Nonetheless, we believe that TPD54 may be a useful and promising marker for the differential diagnosis of salivary gland tumors.
Conclusions

In the TPD52 family, immunohistochemical staining for TPD54 may hold the potential for the pathological differential diagnosis of benign and malignant salivary gland tumors.

Ethical approval

This study was conducted in accordance with the Declaration of Helsinki and was approved by our institutional review board.

Conflict of interests disclosure

None.

References

1. Abdulrahman SS, Mohammad DN, Hamied MA, et al. Immunohistochemical evaluation of salivary gland tumors differentiation and proliferation by using calponin and telomerase. Saudi Dent J. 2019;31:105–114.
2. Abd Raboh NM, Hakim SA. Diagnostic role of DOG1 and p63 immunohistochemistry in salivary gland carcinomas. Int J Clin Exp Pathol. 2015;8:9214–9222.
3. de Paula F, Teshima THN, Hsieh R, et al. Overview of human salivary glands: highlights of morphology and developing processes. Anat Rec (Hoboken). 2017;300:1180–1188.
4. Nagao T, Sato E, Inoue R, et al. Immunohistochemical analysis of salivary gland tumors: application for surgical pathology practice. Acta Histochem Cytochem. 2012;45:269–282.
5. Griffith CC, Schmitt AC, Little JL, et al. New developments in salivary gland pathology: clinically useful ancillary testing and new potentially targetable molecular alterations. Arch Pathol Lab Med. 2017;141:381–395.
6. Byrne JA, Balleine RL, Fejzo MS, et al. Tumor protein D52 (TPD52) is overexpressed and a gene amplification target in ovarian cancer. Int J Cancer. 2005;117:1049–1054.
7. Boutros R, Byrne JA. D53 (TPD52L1) is a cell cycle-regulated protein maximally expressed at the G2-M transition in breast cancer cells. Exp Cell Res. 2005;310:152–165.
8. Mukudai Y, Kondo S, Fujita A, et al. Tumor protein D54 is a negative regulator of extracellular matrix-dependent migration and attachment in oral squamous cell carcinoma-derived cell lines. Cell Oncol (Dordr). 2013;36:223–245.
9. Zhuang Y, Ly RC, Frazier CV, et al. The novel function of tumor protein D54 in regulating pyruvate dehydrogenase and metformin cytotoxicity in breast cancer. Cancer Metab. 2019;7:1. (accessed 2021 May 21) Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6345044/pdf/40170_2018_Article_193.pdf
10. Boutros R, Fanayan S, Shehata M, et al. The tumor protein D52 family: many pieces, many puzzles. Biochem Biophys Res Commun. 2004;325:1115–1121.
11. Ito C, Mukudai Y, Itose M, et al. Tumor proteins D52 and D54 have opposite effects on the terminal differentiation of chondrocytes. Biomed Res Int. 2017;2017:6014278. (accessed 2021 May 21) Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5535702/pdf/BMRI2017-6014278.pdf
12. Kato K, Mukudai Y, Motohashi H, et al. Opposite effects of tumor protein D (TPD) 52 and TPD54 on oral squamous cell carcinoma cells. Int J Oncol. 2017;50:1634–1646.
13. Motohashi H, Mukudai Y, Ito C, et al. Tumor protein D52 expression is post-transcriptionally regulated by T-cell intercellular antigen (TIA) 1 and TIA-related protein via mRNA stability. Biochem J. 2017;474:1669–1677.
14. Yin LX, Ha PK. Genetic alterations in salivary gland cancers. Cancer. 2016;122:1822–1831.
15. Cavalcante RB, Lopes FF, Ferreira AS, et al. Immunohistochemical expression of vimentin, calponin and HHF-35 in salivary gland tumors. Braz Dent J. 2007;18:192–197.
16. Namboodiripad PCA. A review: immunological markers for malignant salivary gland tumors. J Oral Biol Craniofac Res. 2014;4:127–134.
17. Cizkova K, Foltynkova T, Gachechiladze M, et al. Comparative analysis of immunohistochemical staining intensity determined by light microscopy, ImageJ and QuPath in placental Hofbauer cells. Acta Histochem Cytochem. 2021;54:21–29.
18. Ellis GL, Auclair PL. Mucoepidermoid carcinoma. In Atlas of tumor pathology: tumors of the salivary glands. 3rd series fasci 17. Washington DC: Armed Forces Institute of Pathology; 1996. pp155–172.
19. Pinkston JA, Cole P. Incidence rates of salivary gland tumors differentiation and proliferation by using cal- ponin and telomerase. Saudi Dent J. 2016;30149. (accessed 2021 May 21) Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4945913/pdf/srep30149.pdf
20. Yu C, Song Z, Xiao Z, et al. Mucoepidermoid carcinoma arising in Warthin’s tumor of the parotid gland: clinicopathological characteristics and immunophenotypes. Sci Rep. 2016;6:30149. (accessed 2021 May 21) Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4945913/pdf/srep30149.pdf
21. Thomas DDH, Frey CL, Messenger SW, et al. A role for tumor protein TPD52 phosphorylation in endo- membrane trafficking during cytokinesis. Biochem Biophys Res Commun. 2010;402:583–587.
22. Laroque G, La-Borde PJ, Clarke NL, et al. Tumor protein D54 defines a new class of intracellular transport vesicles. J Cell Biol. 2020;219:e201812044. (accessed 2021 May 21) Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7039206/pdf/JCB_201812044.pdf
23. Byrne JA, Tomasetto C, Garnier JM, et al. A screening method to identify genes commonly over-
expressed in carcinomas and the identification of a novel complementary DNA sequence. Cancer Res. 1995;55:2896-2903.

24. Byrne JA, Mattei MG, Basset P. Definition of the tumor protein D52 (TPD52) gene family through cloning of D52 homologues in human (hD53) and mouse (mD52). Genomics. 1996;35:523-532.

25. Byrne JA, Nourse CR, Basset P, et al. Identification of homo- and heteromeric interactions between members of the breast carcinoma-associated D52 protein family using the yeast two-hybrid system. Oncogene. 1998;16:873-881.

26. Cao Q, Chen J, Zhu L, et al. A testis-specific and testis developmentally regulated tumor protein D52 (TPD52)-like protein TPD52L3/hD55 interacts with TPD52 family proteins. Biochem Biophys Res Commun. 2006;344:798-806.

27. Groblewski GE, Yoshida M, Yao H, et al. Immunolocalization of CRHSP28 in exocrine digestive glands and gastrointestinal tissues of the rat. Am J Physiol. 1999;276:G219-G226.

28. Thomas DDH, Weng N, Groblewski GE. Secretagogue-induced translocation of CRHSP-28 within an early apical endosomal compartment in acinar cells. Am J Physiol Gastrointest Liver Physiol. 2004;287:G253-G263.

29. Choschzick M, Lassen P, Lebeau A, et al. Amplification of 8q21 in breast cancer is independent of MYC and associated with poor patient outcome. Mod Pathol. 2010;23:603-610.

30. Ren L, Chen J, Zhang X. Increased expression of tumor protein D54 is associated with clinical progression and poor prognosis in patients with prostate cancer. Oncol Lett. 2017;14:7739-7744.