Current Trends in Immunology
Vol. 24, 2023

Mini-Review

Targeting the stage-specific embryonic antigen (SSEA)-0 tumor neoantigen

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ABSTRACT

Recognition of abnormal glycosylation in virtually any cancer type has raised a great interest in the glycan-based tumor biomarkers. Our team explored carbohydrate microarrays as a broad-spectrum immunoassay to probe the immunologically potent tumor glycan targets. This effort has led to the identification of a blood group precursor antigen SSEA-0 as a conserved breast cancer (BCA) marker. Since this immunogenic O-core glycan is normally hidden as a cryptic antigen but becomes overexpressed and surface-exposed by metastatic breast cancer cells (MBCA), its potential as a novel immunological target for precision immunotherapy against tumor metastasis warrants a focused investigation.

KEYWORDS: abnormal glycosylation, blood group precursors, breast cancer, breast circulating tumor cells, carbohydrate microarray, SSEA-0, tumor glycan marker.

INTRODUCTION

BCA is among the most prevalent cancers and accounts for the highest number of cancer-related deaths among women worldwide [1]. Since tumor metastasis is the main cause of BCA-associated death [2, 3], global efforts are underway to develop targeted tumor immunotherapies to treat metastatic diseases. Notably, these include recent developments in immune checkpoint blockade (ICB) therapy, which activates the intrinsic T-cell immunity to kill the tumor cells [4, 5]. The combination of cancer immunotherapy based on PD-1/PD-L1 immune checkpoint inhibitors (ICIs) with chemotherapy was found effective both in advanced and early setting of Phase 3 clinical trials of BCA treatment [6-8]. These encouraging results have led to the first approval of ICIs for the treatment of triple-negative breast cancer (TNBC) by the United States Food and Drug Administration (FDA) [4].

Current challenges

Despite this progress, challenges remain to the development of highly effective tumor immunotherapies. Unfortunately, many patients are poorly responsive to the current immunotherapy strategies, including ICB and other treatments. Most of the non-responders experience unsuppressed disease progression with poor clinical outcomes [9]. Additionally, ICB therapy may cause severe side effects, including long-term effects, such as immune-related adverse events (irAE) [10, 11]. An open question is how to maximize the ICB-mediated tumor killing effect and minimize the undesired autoimmune responses.

Conceptually, the ICB strategy relies on the intrinsic T-cell immunity to kill the tumor cells [4, 5]. In fact, tumor immunogenicity has been recognized as a key factor in the success of ICB treatment. Although evolved from normal cells, the malignantly transformed tumor cells may express neoantigens

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that are potentially immunogenic. Tumor mutational burden (TMB) has been explored as a prognostic marker of tumor foreignness and immunogenicity. Immunogenic melanoma is a prominent example of ICB success, which has achieved about a 50% response rate in dual anti-PD-1 and anti-CTLA-4 regimens [9, 12].

Although BCA is historically considered poorly immunogenic, recent studies have shown that BCA is highly heterogenic with the mutational load variable among subtypes of BCA. The BCA tumors that present high TMB may include but are not limited to, TNBC and human epidermal growth factor receptor 2 (HER2)-positive BCA [13, 14]. There are growing numbers of pre-clinical and clinical trial investigations focusing on immune therapeutic strategies for BCA patients [6-8].

Immune recognition of tumor-associated neoantigens is evidenced by the presence of tumor-infiltrating lymphocytes (TILs) in the tumor microenvironment (TME). The presence of TILs is associated with a better prognosis and response to chemotherapy, especially when using a combination of ICB treatment and chemotherapy [15]. The immune response captured through immune-related tumor gene expression profiling analyses also demonstrated that some immune gene signatures were associated with a favorable clinical outcome in TNBC and HER2-positive BCA [13, 16]. Using immunophenotyping analyses or transcriptomic approaches, different immune cell subsets have been identified in the TME [17]. The presence of a cytotoxic $CD8^+$ T-cell subpopulation that can kill cancer cells is associated with improved survival in patients, whereas the presence of immunosuppressive regulatory $CD4^+$ T cells (Tregs) or macrophages is associated with a worse prognosis [17].

The tumor neoantigen-targeted immunotherapy strategies include not only therapeutic antibodies but also emerging platforms of cell therapy, such as the chimeric antigen receptor (CAR)-T and T-cell receptor (TCR) engineering therapies [18, 19]. Using engineered antibody binding sites, the CAR-T cells specifically recognize the surface-displayed B-cell epitopes, and TCR therapy targets the major histocompatibility-complex (MHC)-presented tumor peptide epitopes to yield tumor-specific cytotoxicity.

Responding strategies

A pressing need for advancing current immunotherapy approaches is to identify tumor-specific neoantigen to enable precision tumor immunotherapy with minimal or no off-target side effects. Research efforts in this area have led to the identification of several classes of tumor antigens. These include the tumor-specific antigens that are unique for a specific tumor and the tumor-associated antigens (TAAs) that are overexpressed or abnormally produced by tumor cells. It is interesting to note that a class of embryonic proteins, the cancer-testis antigens (CTAs) [20] has been identified as TAAs. Some CTAs have served as targets in vaccination in patients with TNBC [21]. These proteins are normally expressed in embryonic stem cells and testicular germ cells, minimally expressed in most other normal tissues, but are expressed at high levels in many different tumors.

Tumor-associated carbohydrate antigens (TACAs) are another important class of tumor immunological targets [22, 23] that may be explored to develop either passive immunotherapy or active vaccination strategies to combat tumor metastasis. Our team has made a focused effort to identify cell-surface glycan markers of breast circulating tumor cells (bCTCs) and cancer stem cells (bCSCs) [24-26]. Of note, CTCs and CSCs emerge *in vivo* as "mobile" tumor cells in patients with aggressive cancer; these cells are rare but spur metastasis [27, 28]. Detection of CTCs has been explored as a non-invasive "liquid biopsy" for tumor diagnosis and determination of prognosis [29-31]. The CSCs belong to a subpopulation of undifferentiated tumor cells with embryonic characteristics [32-35] and possess epithelial-to-mesenchymal transition traits. As such, CSCs can escape the primary tumor and enter the bloodstream as a subset of CTCs [35, 36]. To date, the lack of tumor-specific cell-surface biomarkers for CTCs and CSCs has limited the development of targeted cancer immunotherapies to eradicate tumor metastasis.

To overcome this difficulty, we used a combination of carbohydrate microarrays and the Fiber-optic Array Scanning Technology (FAST-scan) to explore cell-surface glycan markers of bCTCs and bCSCs. In carbohydrate microarray analyses, we discovered that the SSEA-0 neoantigen is specifically

recognized by an anti-tumor monoclonal antibody (mAb) HAE3 [24, 37]. Since a subclone C1 of hybridoma HAE3 was used in our recent studies, we precisely defined the antibody targeting specificity by identifying the glycan epitope C1 (gp^{C1}) [24, 25, 38].

Applying gp^{C1}-specific mAbs, we examined SSEA-0 expression in bCTCs or bCSCs using FAST-scan and other immune assays. In a clinical case study [24], blood samples from five patients with Stage IV MBCA were characterized by FAST-scan. The SSEA-0/gp^{C1+}-CTCs were detected in all subjects with~ 40% of bCTCs strongly positive. Interestingly, the CTCs from a patient with TNBC and multiple sites of metastasis were predominantly SSEA-0/gp^{C1} positive (92.5%, 37/40 CTCs). Using fluorescence-activated cell sorting (FACS) cell-surface staining, we characterized gp^{C1}-distribution in a panel of CSC-like BCA lines. These included the human TNBC line BT-549, Hs 578T, MDA-MB-231, and MDA-MB-468. Overexpression of SSEA-0/gp^{C1} among these cells was detected by FACS. The BT-549 and MDA-MB-231 were strongly SSEA-0/gp^{C1} positive. The MDA-MB-468 was intermediately positive. The Hs 578T cells were, however, generally negative except for a small set of weakly positive cells [37]. Additionally, we examined the breast tissue distribution of SSEA-0/gp^{C1} using a set of tissue microarrays (TMAs). 44/78 (56%) neoplastic breast tissues including the major histological types of BCA were found to be SSEA-0/gp^{C1} positive. Conversely, none of the breast tissues derived from subjects without BCA were gp^{C1} positive [39]. Thus, SSEA-0/gp^{C1} appears to be a conserved marker expressed by the BCA cells that are highly metastatic.

Glycan structure of SSEA-0 and its biosynthesis

Figure 1 is a schematic of the SSEA-0/gp^{C1} determinant. It belongs to the blood group precursor cores in the histo-blood group antigen system [24, 37, 40, 41]. This complex core structure was proposed based on extensive glycan structural analyses and immunochemical studies of a large collection of native blood group substances [41-44]. The four-branched structure in the circle represents the internal portion of the carbohydrate moiety of blood group substances. The terminal nonreducing β-galactoside epitopes of Iβ [Galβ1, 3GlcNAcβ1,6)] and/or IIβ [(Galβ1,4GlcNAcβ1,6)] are crucial for preserving the potent SSEA-0/gp^{C1} determinant(s). Fucosylation of these internal domains or cores blocks surface exposure of the SSEA-0/gp^{C1} cryptic epitope(s) but results in mature blood group antigens, such as A, B, O, and the Lewis (Le) series of antigens.

Several SSEA antigens in the histo-blood group system have been found to be overexpressed in BCA. These include blood group I antigen [45-47], SSEA-1/Le^X [48-50], and SSEA-5/H type-I [51]. The SSEA-1/Le^X and SSEA-5/H-type I are fucosylated "matured" blood group antigens derived from the blood group precursor cores (Figure 1). The I-antigen is formed by polylactosamine chain with branches. However, these blood group antigens are HAE3-negative [24, 37, 52]. Unlike other SSEA antigens, which are often co-expressed by normal cell types, SSEA-0 is cryptic in normal cells not accessible for immune recognition. Nevertheless, this target appears to be immunogenic upon its tumor expression. HAE3 was in fact raised against epiglycanin, the major sialomucin glycoprotein (~ 500 kDa) of murine mammary adenocarcinoma TA3 cells [53]. Targeting such conserved cryptic tumor antigen may be explored as a unique immunotherapy strategy to achieve precision tumor-killing without autoimmune cross-reactivity.

The key glyco-enzymes involved in the biosynthesis of blood group precursor have been identified. These include β-1,6-N-acetylglucosaminyltransferases (GCNT1, 2, and 3) that catalyze β-1,6-branching of the O-cores and the polylactosamine extension enzyme (B3GNT2) (noted in Figure 1). Interestingly, gene-expression analysis by other researchers recognized BCA-associated overexpression of these glycogenes. GCNT2 is crucial for this biosynthesis pathway and its overexpression was detected in highly metastatic BCA cell lines of mouse or human origin and in clinical basal-like breast tumor samples [54-56]. Ectopic expression of GCNT2 was found to enhance cell detachment, adhesion to endothelial cells, cell migration, invasion *in vitro*, and lung metastasis of breast cancer cells *in vivo*. Knockdown of GCNT2 expression in fact decreased cell migration and invasion *in vitro* and

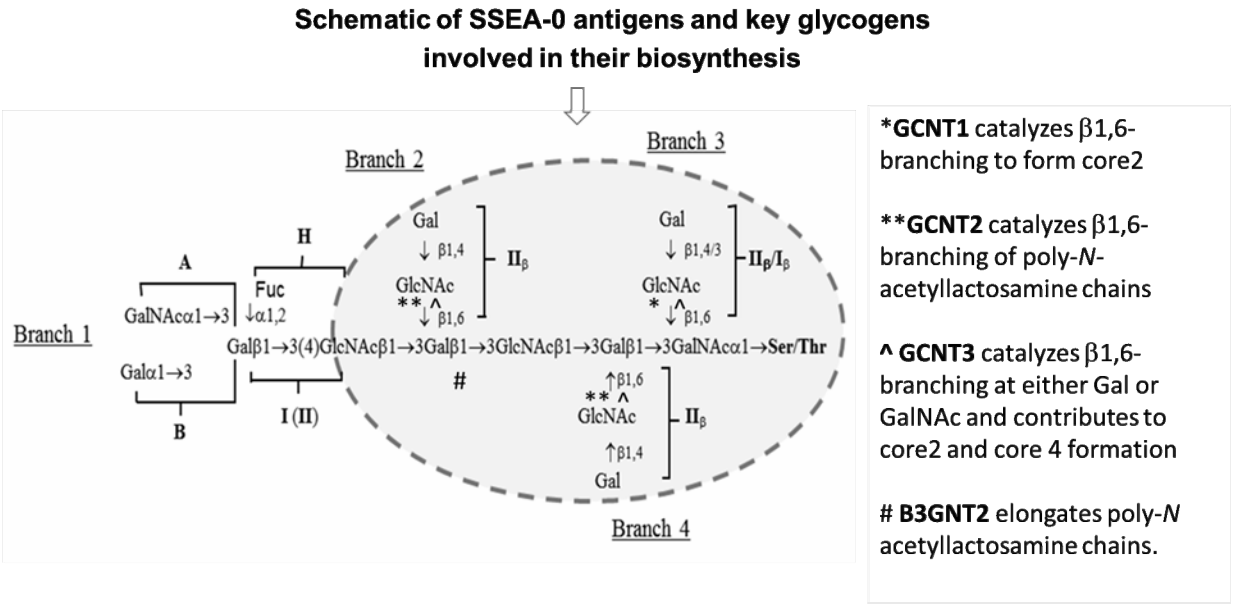

Figure 1. A schematic of SSEA-0/gp^{C1} blood group precursor antigens and key enzymes for their biosynthesis. Blood group precursors are characteristically composed of oligosaccharide chains with branches of IIβ (Galβ1,4GlcNAcβ1,6) or Iβ (Galβ1,3GlcNAcβ1,6)-moieties without fucosylation. As noted, key glycogenes involved in SSEA-0 biosynthesis may include GCNT1/2/3 that catalyze β-1,6-branching of the O-cores and B3GNT2, commonly required for blood group I synthesis. Anti-gp^{C1} antibodies have minimal or no cross reactivity with conventional I-blood group antigens, highlighting the structural diversity of the blood group precursor-based oncofetal antigens.

lung metastasis *in vivo*. Thus, it is not impossible to induce tumor expression of such immunogenic carbohydrates *via* epigenomic modulation of glycogen expression to trigger intrinsic antitumor immunity.

Conclusion remarks

There is compelling evidence to identify the SSEA-0 neoantigen as a potent immunological target in BCA. Since this target is normally hidden as a cryptic antigen but becomes overexpressed and surface exposed by MBCA cells, its potential as a novel immunological target for the early detection of MBCA and precision immunotherapy against tumor metastasis warrants further investigation. It is noteworthy that the HAE3 positive tumor antigens were previously termed as human carcinoma-associated antigens (HCA), which were found to be over-expressed by other human epithelial tumors, including lung, prostate, bladder, esophagus, and ovarian cancers [57-59]. Thus, the SSEA-0-targeting therapeutic strategies may be effective therapeutics for additional epithelial cancers.

ACKNOWLEDGMENTS

This work was supported in part by US Government grants U01CA128416 (NIH), R21CA254048 (NIH), and PR210339 (CDMRP) to DW. The content is solely the responsibility of the author and does not necessarily represent the official views of the funding agents.

CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest to declare for any of the authors involved in this work.

REFERENCES

1. Chatterjee, S. K. and Zetter, B. R. 2005, Future Oncol., 1, 37.
2. Siegel, R. L., Miller, K. D., Fuchs, H. E. and Jemal, A. 2022, CA Cancer J. Clin., 72, 7.
3. Giaquinto, A. N., Sung, H., Miller, K. D., Kramer, J. L., Newman, L. A., Minihan, A., Jemal, A. and Siegel, R. L. 2022, CA Cancer J. Clin., 72, 524.

4. Debien, V., De Caluwé, A., Wang, X., Piccart-Gebhart, M., Tuohy, V. K., Romano, E. and Buisseret, L. 2023, npj Breast Cancer, 9, 7.
5. Barzaman, K., Moradi-Kalbolandi, S., Hosseinzadeh, A., Kazemi, M. H., Khorramdelazad, H., Safari, E. and Farahmand, L. 2021, Int. Immunopharmacol., 98, 107886.
6. Solinas, C., Garaud, S., De Silva, P., Boisson, A., Van den Eynden, G., de Wind, A., Risso, P., Rodrigues Vitoria, J., Richard, F., Migliori, E., Noel, G., Duvillier, H., Craciun, L., Veys, I., Awada, A., Detours, V., Larsimont, D., Piccart-Gebhart, M. and Willard-Gallo, K. 2017, Front Immunol, 8, 1412.
7. Solinas, C., Gombos, A., Latifyan, S., Piccart-Gebhart, M., Kok, M. and Buisseret, L. 2017, ESMO Open, 2, e000255.
8. Adams, S., Diamond, J. R., Hamilton, E., Pohlmann, P. R., Tolaney, S. M., Chang, C. W., Zhang, W., Iizuka, K., Foster, P. G., Molinero, L., Funke, R. and Powderly, J. 2019, JAMA Oncol., 5, 334.
9. Mellman, I., Coukos, G. and Dranoff, G. 2011, Nature, 480, 480.
10. Emens, L. A., Adams, S., Cimino-Mathews, A., Disis, M. L., Gatti-Mays, M. E., Ho, A. Y., Kalinsky, K., McArthur, H. L., Mittendorf, E. A., Nanda, R., Page, D. B., Rugo, H. S., Rubin, K. M., Soliman, H., Spears, P. A., Tolaney, S. M. and Litton, J. K. 2021, J Immunother Cancer, 9(8), e002597.
11. Ghisoni, E., Wicky, A., Bouchaab, H., Imbimbo, M., Delyon, J., Gautron Moura, B., Gerard, C. L., Latifyan, S., Ozdemir, B. C., Caikovski, M., Pradervand, S., Tavazzi, E., Gatta, R., Marandino, L., Valabrega, G., Aglietta, M., Obeid, M., Homicsko, K., Mederos Alfonso, N. N., Zimmermann, S., Coukos, G., Peters, S., Cuendet, M. A., Di Maio, M. and Michielin, O. 2021, Eur. J. Cancer, 149, 153.
12. Larkin, J., Chiarion-Sileni, V., Gonzalez, R., Grob, J. J., Rutkowski, P., Lao, C. D., Cowey, C. L., Schadendorf, D., Wagstaff, J., Dummer, R., Ferrucci, P. F., Smylie, M., Hogg, D., Hill, A., Marquez-Rodas, I., Haanen, J., Guidoboni, M., Maio, M., Schoffski, P., Carlino, M. S., Lebbe, C., McArthur, G., Ascierto, P. A., Daniels, G. A., Long, G. V., Bastholt, L., Rizzo, J. I., Balogh, A., Moshyk, A., Hodi, F. S. and Wolchok, J. D. 2019, N Engl. J. Med., 381, 1535.
13. Desmedt, C., Haibe-Kains, B., Wirapati, P., Buyse, M., Larsimont, D., Bontempi, G., Delorenzi, M., Piccart, M. and Sotiriou, C. 2008, Clin. Cancer Res., 14, 5158.
14. Bareche, Y., Buisseret, L., Gruosso, T., Girard, E., Venet, D., Dupont, F., Desmedt, C., Larsimont, D., Park, M., Rothe, F., Stagg, J. and Sotiriou, C. 2020, J. Natl. Cancer Inst., 112, 708.
15. Savas, P., Salgado, R., Denkert, C., Sotiriou, C., Darcy, P. K., Smyth, M. J. and Loi, S. 2016, Nat. Rev. Clin. Oncol., 13, 228.
16. Teschendorff, A. E., Miremadi, A., Pinder, S. E., Ellis, I. O. and Caldas, C. 2007, Genome Biol., 8, R157.
17. Allard, B., Aspeslagh, S., Garaud, S., Dupont, F.A., Solinas, C., Kok, M., Routy, B., Sotiriou, C., Stagg, J. and Buisseret, L. 2018, Semin Cancer Biol., 52, 1.
18. Sadelain, M., Rivière, I. and Riddell, S. 2017, Nature, 545, 423.
19. Wachsmann, T. L. A., Wouters, A. K., Remst, D. F. G., Hagedoorn, R. S., Meeuwsen, M. H., van Diest, E., Leusen, J., Kuball, J., Falkenburg, J. H. F. and Heemskerk, M. H. M. 2022, Oncoimmunology, 11, 2033528.
20. Wang, C., Gu, Y., Zhang, K., Xie, K., Zhu, M., Dai, N., Jiang, Y., Guo, X., Liu, M., Dai, J., Wu, L., Jin, G., Ma, H., Jiang, T., Yin, R., Xia, Y., Liu, L., Wang, S., Shen, B., Huo, R., Wang, Q., Xu, L., Yang, L., Huang, X., Shen, H., Sha, J. and Hu, Z. 2016, Nat Commun., 7, 10499.
21. Lam, R. A., Tien, T. Z., Joseph, C. R., Lim, J. X., Thike, A. A., Iqbal, J., Tan, P. H. and Yeong, J. P. S. 2021, Cancers (Basel), 13(15), 3875.
22. Feng, D., Shaikh, A. S. and Wang, F. 2016, ACS Chemical Biology, 11, 850.
23. Wang, D. 2014, J. Proteomics Bioinform, 7(2), 23539.
24. Wang, D., Liu, X., Hsieh, B., Bruce, R., Somlo, G., Huang, J. and Sambucetti, L. 2015, Arch. Med. Res., 46, 642.

25. Wang, D. 2017, J. Proteomics Bioinform, 10(1), e31.
26. Jiang, Y. and Wang, D. 2018, Open Access J. Biomed. Eng. Appl., 1(3), 115.
27. Jacob, K., Sollier, C. and Jabado, N. 2007, Expert review of proteomics, 4, 741.
28. Hayashi, N. and Yamauchi, H. 2012, Breast Cancer, 19, 110.
29. Somlo, G., Lau, S. K., Frankel, P., Hsieh, H. B., Liu, X., Yang, L., Krivacic, R. and Bruce, R. H. 2011, Breast cancer research and treatment, 128, 155.
30. Das, M., Riess, J. W., Frankel, P., Schwartz, E., Bennis, R., Hsieh, H. B., Liu, X., Ly, J. C., Zhou, L., Nieva, J. J., Wakelee, H. A. and Bruce, R. H. 2012, Lung Cancer, 77, 421.
31. Liu, X., Hsieh, H. B., Campana, D. and Bruce, R. H. 2012, Cytometry A, 81, 169.
32. Lapidot, T., Sirard, C., Vormoor, J., Murdoch, B., Hoang, T., Caceres-Cortes, J., Minden, M., Paterson, B., Caligiuri, M. A. and Dick, J. E. 1994, Nature, 367, 645.
33. Al-Hajj, M., Wicha, M. S., Benito-Hernandez, A., Morrison, S. J. and Clarke, M.F. 2003, Proc. Natl. Acad. Sci. USA, 100, 3983.
34. Chiotaki, R., Polioudaki, H. and Theodoropoulos, P. A. 2015, Curr. Cancer Drug Targets, 15, 256.
35. de Beca, F. F., Caetano, P., Gerhard, R., Alvarenga, C. A., Gomes, M., Paredes, J. and Schmitt, F. 2013, J. Clin. Pathol., 66, 187.
36. Karsten, U. and Goletz, S. 2013, Springerplus, 2, 301.
37. Wang, D., Tang, J., Liu, S. and Huang, J. 2015, Journal of immunology research, 2015, 510810.
38. Wang, D., Wu, L. and Liu, X. 2017, Advances in experimental medicine and biology, 994, 275.
39. Jiang, Y. and Wang, D. 2020, Int. J. Cancer Sci. Ther., 2(1), 10.31487/j.ijcst.2020.01.04.
40. Maisonrouge-McAuliffe, F. and Kabat, E. A. 1976, Arch Biochem Biophys, 175, 71.
41. Vicari, G. and Kabat, E. A. 1970, Biochemistry, 9, 3414.
42. Feizi, T., Kabat, E. A., Vicari, G., Anderson, B. and Marsh, W. L. 1971, J. Immunol., 106, 1578.
43. Feizi, T., Kabat, E. A., Vicari, G., Anderson, B. and Marsh, W. L. 1971, The Journal of experimental medicine, 133, 39.
44. Wu, A. M., Khoo, K. H., Yu, S. Y., Yang, Z., Kannagi, R. and Watkins, W. M. 2007, Proteomics, 7, 3699.
45. Burchell, J., Wang, D. and Taylor-Papadimitriou, J. 1984, Int. J. Cancer, 34, 763.
46. Dube, V. E., Haid, M., Chmiel, J. S. and Anderson, B. 1984, Breast cancer research and treatment, 4, 105.
47. Dube, V. E., Kallio, P., Chmiel, J. S., Haid, M. and Hakim, A. 1987, Clin. Immunol. Immunopathol., 45, 196.
48. Koh, Y. W., Lee, H. J., Ahn, J. H., Lee, J. W. and Gong, G. 2013, Am. J. Clin. Pathol., 139, 746.
49. Renkonen, J., Paavonen, T. and Renkonen, R. 1997, Int. J. Cancer, 74, 296.
50. Nakagoe, T., Fukushima, K., Itoyanagi, N., Ikuta, Y., Oka, T., Nagayasu, T., Ayabe, H., Hara, S., Ishikawa, H. and Minami, H. 2002, J. Cancer Res. Clin. Oncol., 128, 257.
51. Tang, C., Lee, A. S., Volkmer, J. P., Sahoo, D., Nag, D., Mosley, A. R., Inlay, M. A., Ardehali, R., Chavez, S.L., Pera, R. R., Behr, B., Wu, J. C., Weissman, I. L. and Drukker, M. 2011, Nat. Biotechnol., 29, 829.
52. Palma, A. S., Liu, Y., Childs, R. A., Herbert, C., Wang, D., Chai, W. and Feizi, T. 2011, Biochemical and biophysical research communications, 408, 548.
53. Codington, J. F., Sanford, B. H. and Jeanloz, R. W. 1972, Biochemistry, 11, 2559.
54. Zhang, H., Meng, F., Wu, S., Kreike, B., Sethi, S., Chen, W., Miller, F. R. and Wu, G. 2011, Cancer Res., 71, 4846.
55. Potapenko, I. O., Haakensen, V. D., Luders, T., Helland, A., Bukholm, I., Sorlie, T., Kristensen, V. N., Lingjaerde, O. C. and Borresen-Dale, A. L. 2010, Mol. Oncol., 4, 98.

56. Potapenko, I. O., Luders, T., Russnes, H. G., Helland, A., Sorlie, T., Kristensen, V. N., Nord, S., Lingjaerde, O. C., Borresen-Dale, A. L. and Haakensen, V. D. 2015, Mol. Oncol., 9, 861.
57. Li, R., Yao, J. L., Bourne, P. A., di Sant'Agnese, P. A. and Huang, J. 2004, Arch. Pathol. Lab. Med., 128, 1412.
58. Liang, S., Yao, J., Bourne, P. A., diSant'Agnese, P. A., Huang, J. and Lei, J.Y. 2004, Am. J. Clin. Pathol., 122, 747.
59. Yao, J. L., Bourne, P. A., Yang, Q., Lei, J., di Sant'Agnese, P. A. and Huang, J. 2004, Arch. Pathol. Lab Med., 128, 785.