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A STUDY OF BIOCHEMICAL CHARACTERIZATION OF ANTIGEN RECOGNIZED BY MONOCLONAL ANTIBODY CAMA3C8 AGAINST HUMAN BREAST CANCER CELL LINE

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Abstract

Biochemical studies have shown structural alterations in carbohydrates present on cell membrane constituents like glycolipids, glycoprotein and cell secretary products accompanying neoplastic transformation. Many of the monoclonal antibodies raised against cancer cells have been shown to react with these alternated carbohydrates. Therefore, these monoclonal antibodies are useful for immunohistochemical diagnosis of cancer in specific organ. In the present study, the biochemical characterization of antigen recognized by monoclonal antibody CAMA3C8 against human breast cancer cell line was investigated. The study was conducted at Meenakshi Medical College Hospital and Research Institute, Kanchipuram, Tamil Nadu, India. Immuno histochemical method was used to determine whether the antigenic determinant is a carbohydrate moiety or not. This is a combination of an avidin-biotin- peroxidase complex method and acetylation technique. There was no positive reaction product for CAMA3C8 was found in the acetylated CAMA cells. The positive reaction reappeared in deacetylated cells similar to the peroxidase staining. This technique was used to confirm that monoclonal antibody CAMA3C8 is specific for an epitope on the carbohydrate moiety of the antigenic molecule. We conclude that the CAMA3C8 recognizes an antigen which is highly glycated with O-linked carbohydrate moiety with N-terminal neuraminic acid in breast cancer cell line.

INTRODUCTION

Biochemical studies have classified glycoproteins according to the nature, link between the oligosaccharide chains and polypeptides. Two families have been considered: N and O-linked glycoproteins. N- glycosidic bond is established between N-acetyl glucosamine (GLUNAC) and the amide nitrogen of asparagines (GLUNAC [β1-N] Asn). The O-glycosidic bond presents a variety of links, the most important being the mucin type in which the oligosaccharide chains are linked from N-acetyl-D-galactosamine to the hydroxyl groups of L-Serine and or L-Threonine (N- Acetyl Galactosamine) (α1,3) serine or (N- Acetyl Galactosamine) (α1,3) Threonine) [1,2] .

Biochemical properties of tumor associated antigen (TAA) have been extensively studies by application of Monoclonal antibodies. In this study monoclonal antibody (MAb) CAMA3C8 has been generated using human breast carcinoma cell line CAMA as an immunogen. A preliminary study showed the presence of CAMA3C8 antigen in sera of cancer patients. The distribution of the antigen recognized by CAMA3C8 in various cancers and normal tissues has been studied immunohistochemistry [3].

MATERIALS AND METHODS

The study was conducted at Meenakshi Medical College Hospital and Research Institute, Kanchipuram, Tamil Nadu, India. The study was performed after obtaining approval from the institutional ethical committee.

Cell line

Human breast carcinoma cell line – CAMA was cultured in Dulbecco`s modified Eagle`s medium supplemented with 10% fetal calf serum, 2mM glutamine, 100 IU/ml penicillin, 50µg/ml streptomycin.

Isolation of MAb CAMA3C8 Defined Antigen

Crude membrane extracts were prepared from cultured CAMA cells. The crude membrane fractions of CAMA cells were subjected to PCA precipitation at 4°C using the method of Delong and Davidson (1981) [4]. 5ml of crude membrane fractions of CAMA cells were chilled in ice and an equal volume of 1.2M perchloric acid (PCA) added drop wise with stirring. The mixture was incubated in an ice bath for 20min with occasional agitation and centrifuged at 10,000rpm for 10min. The pellet was discarded and the supernatant neutralized by the addition of 1.2MKOH. The potassium perchlorate was removed by centrifugation at 10,000rpm for 5mins and the supernatant dialyzed against water, lyophilized, stored at -20◦C. Immunoreactivities of this antigenic material was assessed by ELISA using CAMA3C8.

Purification of CAMA3C8 Defined Antigen by Immunoaffinity Chromatography

The affinity column was prepared for the purification of membrane proteins. Antibody was coupled to sephrose (large-pore Chromatography matrix) high molecular – weight antigens pass freely into and out of the pores and bind to antibodies covalently bound to the matrix. In order to elute the bound protein antigen from the immunoaffinity matrix, the antibody antigen interaction is destabilized by brief exposure to low-pH buffer.

Molecular Weight Determination of the CAMA3C8 Defined Antigen

The purified CAMA3C8 antigen was subjected to 5% sodium dodecyl sulphate – polyacrylamide gel electrophoresis (SDS- PAGE).

Glycoprotein Analysis of CAMA3C8 Defined Antigen by PAGE

Purified CAMA3C8 antigen was analyzed for the presence of carbohydrate by Polyacrylamide gel electrophoresis by staining the gel basic fusion and coomassie blue [5].

Identification of the Glycoprotein Epitope on the Antigen

Immunohistochemical method has been used to determine whether the antigenic determinant is a carbohydrate moiety or not using a complementary immunohistochemical method [6] which is a combination of an avidin-biotin- peroxidase complex method [7] and acetylation technique [8].

Three different types of processing of CAMA cells have been employed in this study. The distribution of antigen recognized by this antibody is first determined by the ABC method. By acetylation the binding sites on the cells are masked and by reacetylation only the carbohydrate moiety is exposed. By performing different sets of experiments with acetylation and deacetylation of cells followed by ABC staining, it is possible to identify the binding sites which are carbohydrate in nature.

RESULTS AND DISCUSSION

Results

Isolation of CAMA3C8 defined antigens was carried out from CAMA cell membrane extracts. The glycoprotein was soluble in perchloric acid resulting in 10fold enrichment of the antigen. The crude membrane fraction was subjected to perchloric acid precipitation. The immune reactivity of PCA precipitation fraction was assesses by ELISA using MAb CAMA3C8.

CAMA3C8 defined antigen purification was performed applying the solubilized PCA fraction of CAMA cell membrane extracts to the affinity chromatographic column of sephrose 4B coupled to CAMA3C8 followed by extensive washings and elution at low pH. Antigen activity of the eluate was determined by ELISA. Purification of the antigen was achieved in one step. The purified antigen was concentrated and tested for homogeneity by SDS-PAGE. SDS PAGE was exercised before and after purification (Fig 1, 2). The highly purified antigen showed a single discrete band (Fig 2). This purified antigen was used for further characterization studies.

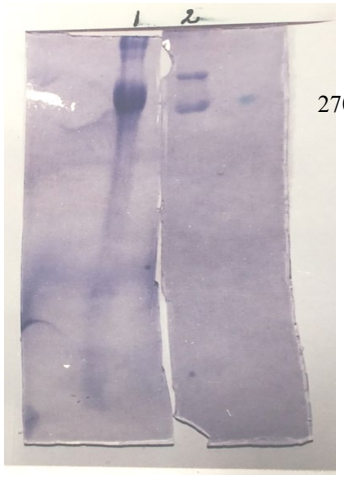

Fig. 1. SDS- PAGE analysis of PCA soluble fraction of CAMA3C8 defined antigen.
Lane 1: CAMA cell membrane of PCA soluble fraction
Lane 2: Molecular weight markers

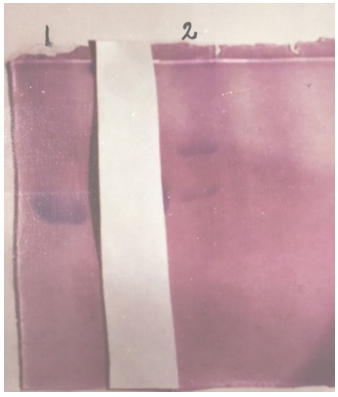

Fig. 2. SDS- PAGE analysis of affinity purified CAMA3C8 defined antigen.
Lane 1: Affinity Eluant
Lane 2: Molecular weight markers

The fractions obtained after affinity chromatographies were concentrated and were analyzed by SDS-PAGE. Fig 3 and Fig 4 illustrates the results obtained after staining the gels with Schiff`s reagent for carbohydrate and coomassic blue for protein. These results indicated the presence of a broad high molecular weight band in this fraction when stained with Schiff`s reagent indicating the glycoprotein nature of this antigen. In the case of staining with coomassie blue the band was very thin with the same molecular weight. The intense staining of the antigenic band with Schiff`s reagent compared to the staining with coomassie blue might indicate that this antigen as a high content of carbohydrate. This might suggest its dramatic elevation in the antigen that the CAMA3C8 MAb recognizes epitopes which are mucin like glycoprotein's expressed in high level in the antigenic extract of CAMA cells.

Fig. 3. CAMA3C8 defined antigen was subjected to SDS-PAGE by using 5% gel. The gel was stained with Schiff`s reagent.

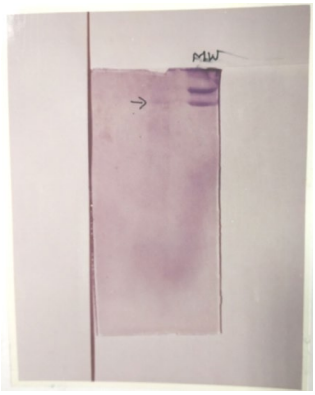

Fig. 4. CAMA3C8 defined antigen was subject to SDS-PAGE by using 5%gel. Gel was then stained with Coomassie Blue.

The antigenic determinants recognized by most MAbs have not been generally clarified. A complementary immunohistochemical method was carried out for detection whether carbohydrate is related to the antigenic determinant or not using a combination of an avidin-biotin complex (ABC) method and acetylation techniques. CAMA3C8 has been demonstrated to reveal membrane staining in CAMA cells by immunoperoxidase method. Although no positive reaction product for CAMA3C8 was found in the acetylated CAMA cells. (Fig.5). The positive reaction reappeared in deacetylated cells similar to the peroxidase staining (Fig 6). This technique was used to confirm that MAb CAMA3C8 is specific for an epitope on the carbohydrate moiety of the antigenic molecule.

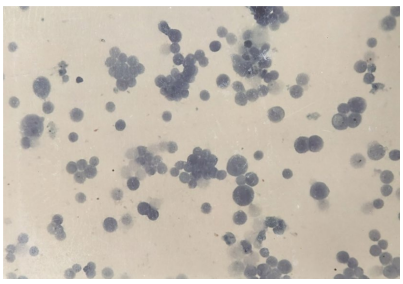

Fig. 5. Acetylated CAMA cells showing no positive reaction with Mab CAMA3C8

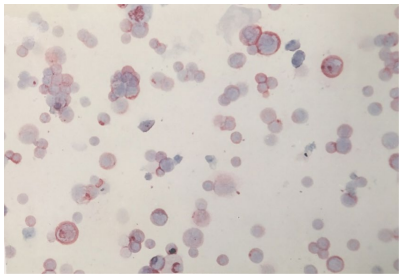

Fig. 6. Deacetylated cells after acetylation showing positive reaction with Mab CAMA3C8

From these studies there is conclusive evidence indicating that the MAb CAMA3C8 recognizes an epitope which is carbohydrate in nature on the antigen molecule and there is high expression of mucin like glycoprotein by CAMA cells as revealed by immunoperoxidase staining of cells and tissue sections.

In order to determine whether CAMA3C8 defined antigen is a protein, a glycoprotein, or glycolipid, biochemical studies like enzyme treatment of the CAMA membrane extracts has been carried out. An ELISA was performed after digestion with various enzymes like trypsin, protease, pronase, hyaluronidase, collagenase and neuraminidase. The antigen binding ratio are represented in (Table 1). Extraction with methanol did not reveal any antigenicity in ELISA indicating that it is not a glycolipid.

The results shown in Table 1 demonstrate that CAMA3C8 defined antigen was also sensitive to digestion with pronase and protease, while trypsin digestion slightly reduced CAMA3C8 binding.

Table 1. Antigen Binding Ratio of 3C8MAb to CAMAlysate antigen after various treatments

S. No.	Treatment	Binding Ratio
1.	None	1.00
2.	Heat	0.93
3.	Neuraminidase	0.12
4.	$NaIO_4$	0.67
5.	0.1N NaOH	0.76
6.	Protease VI	0.30
7.	Trypsin	0.67
8.	Collagenase	0.89
9.	Pronase	0.60
10.	Hyaluronidase	0.90
11.	Methanol	0.90
12.	1.0M Sodium borohydride	0.75

Antigen Binding Ratio based on the average of various concentrations of the enzymic and other modality treatments of CAMA3C8 by Trypsin, Protease, Neuraminidase and Sodium Periodate.

To test whether sialic acid is required for antibody binding, antibody was tested for sensitivity to neuraminidase in the result shown in (Table 1). Binding of antibody CAMA3C8 was also neuramindase sensitive suggesting that sialic acid is required for binding of this antibody taken. This sensitivity of CAMA3C8 defined antigen to neuraminidase suggests that sialic acid is a component of the antigenic determinant or is responsible for maintaining its conformation, and that the CAMA3C8 defined epitope may be expressed on a carbohydrate moiety.

To determine whether carbohydrate structures are required for binding of the antibody, the effects of sodium periodate treatment on antibody binding (Table 1). The binding of CAMA3C8 antibody was sensitive to periodate treatment up to a 65% reduction at 7mM concentration and suggests that carbohydrate is required for binding of this antibody. The antigenicity of carbohydrate chains are easily lost by oxidation with periodic acid. The results of periodic acid and neuraminidase treatments indicate that the antigenic determinant recognized by CAMA3C8 is a carbohydrate chain with terminal sialic acid. Further characterization of the CAMA3C8 defined antigen was performed by heart treatment. The samples were heated to 100◦C for 15min. the antigen has been shown to be resistant to heart treatment, so CAMA3C8 defined antigen is stable at 100◦C for more than 15min.

Further characterization of the CAMA3C8 defined antigen was performed by treating with sodium hydroxide and sodium borohydride (Table 1). The antigen has been shown to be sensitive to alkaline borohydride treatment indicating that it contains O- linked oligosaccharides. Because of this and other characteristic properties of the antigen here after it is referred to as a mucin like antigen.

Discussion

CAMA3C8 is a monoclonal antibody generated against human breast carcinoma cell line – CAMA. Flow cytometric analysis performed to determine the binding of CAMA3C8 to the cell surface antigen of human breast carcinoma cell lines has revealed that this antibody was reactive with the two cell lines CAMA and ZR75 the maximum percentage of positive cells being with ZR75. The antigenic determinants can vary between different sources, as has been shown for a breast cancer associated mucin like antigen by Kothari et al [8]. These investigators showed that the determinants recognized by two monoclonal antibodies HMFG-1 and HMFG-2 differ in their relative abundance depending on the sources of the antigen.

By immunohistochemical studies using indirect techniques like immunoperoxidase test, membrane immunofluorescence study and immune electron microscopic study the CAMA3C8 defined antigen has been found to be present on the cell membrane. For the characterization of the CAMA3C8 reactive antigen, CAMA cells obtained freshly from cultures have been used for the isolation of the antigen.

The molecular weight and homogeneity were tested by SDS- PAGE and showed a single discrete band. The molecular weight of the antigen as determined by SDS-PAGE was Molecular weight 270,000. This was also supported by single band antigen reactivity detected in immunoprecipitation or immunoblotting after transfer on to nitrocellulose paper. The unaltered migration of the protein after reduction by 2-mercaptoethanol suggested that it had no inter or intra molecular disulphide bridges. One of the first objectives in characterizing a MAb defined epitope on mucin molecule is to establish whether the epitope is carbohydrate or peptide in nature. Some epitopes may demonstrate characteristics of both. Several MAbs defined tumor associated epitomes on mucins demonstrate both carbohydrate and protein characteristics [9,10,11]. CAMA3C8 defined antigen appears to be a glycoprotein that stains strongly with PAS and family with coomassie blue, indicating a preponderance of carbohydrate in this molecule. Acetylation techniques were usually combined with periodic acid Schiff (PAS) stain and are useful for the detection of carbohydrates [8]. Positive reaction for CAMA3C8 was removed in deacetylated sections. On the other hand acetylated, 1,2- glycol linkage is returned by deacetylation [9]. This technique is used to confirm the MAb CAMA3C8 specific for carbohydrate antigenic epitope. The immunocytochemical studies using the deacetylation experiments which defined 1-2 glycol linkage oligosaccharide conjugates and that CAMA3C8 recognized carbohydrate epitope.

The degree of glycosylation of this glycoprotein's can be easily accessed by studying the solubility of the antigen in perchloric acid (PCA). When the solubility of the serum type 2 chain plylactosamine antigen, the sialyated Le^x-i antigen,in 0.6N PCA was tested , the results showed that the antigen in the sera of patients with the cancer was more soluble in PCA than the same antigen that occasionally appears in the serum, of patients with benign diseases [15]. CAMA3C8 defined antigen purification has been performed from the solubilized PCA fraction of CAMA cell membrane extracts, this means that CAMA3C8 defined antigen is carried by the glycoproteins that are highly glycosylated. In other words CAMA3C8 defined antigen has high carbohydrate/protein ratio. The antigen typically contains a high proportion of O-linked oligosaccharides with an apparent molecular weight of molecular weight 270,000 and because of their chemical similarities of glycoprotein's found in mucus secretions, are generally referred to as mucin-like [19]. Mucins contain many carbohydrate chains and are differentially glycosylated. This could explain the microheterogenity of the molecule as indicated by the broad bands in gel protein (Fig. 1-2). Since the protein content is much lower than polysaccharide in the mucin molecules. This signal may not be strong enough have confirmed that the antigen is expressed on diffusely migrating high molecular weight glycoproteins a large proportion of which barely enter the 5% gels. These are characteristic properties of highly glycosylated macromolecules such as mucins and proteoglycans.

Biochemical data, suggests that the oligosaccharide side chain and or conformation may play a role in the antigenicity of these antigens [12, 13]. However, oligosaccharide chains in glycoproteins are extremely heterogeneous and digestion of CAMA3C8 defined antigen with protease, trypsin and pronase suggest the ability of the molecule to bind to antibody is either lost when the conformation is changed or

markedly reduced in isolated oligosaccharides. The antigenic activity was affected by these enzyme digestions. Pronase are less specific than trypsin and would generally yield smaller peptide fragments due to greater proteolysis, while CAMA3C8 defined antigen would be more susceptible to proteolysis, since more of the peptide is exposed for cleavage. The loss of CAMA3C8 binding after protease treatment of antigen suggests that the peptide backbone may aid conformationally in the optimal binding of this MAb. Similarly, proteolytic treatment of mucins decreased antigenic activities defined by the MAb B72.3 [18], DUPAN-21[7] and CAL 912) suggesting that the conformational integrity of these epitopes is dependent on peptide.

Enzymes have been used to characterize mucin antigen, chondroitinase ABC, Hyaluronidase and Chondro-4-Sulfatase digestion did not affect relevant MAb binding to TAG-72, DF3 [13,14] and Pancreatic Cancer-Associated Antigen (DU-PAN-2), showing that these determinants are not carried on proteoglycans type oligosaccharides. Similarly the resistance of the CAMA3C8 defined to hyaluronidase suggests that the epitope is not expressed on a proteoglycan. The resistance of CAMA3C8 defined antigen to collagenase and hyaluronidase also indicates that the molecular size and heterogeneity of the CAMA3C8 antigen are not a result of non-covalent interactions with collagen or hyaluronic acid.

The biochemical nature of the CAMA3C8 reactive epitope was further analyzed by neuraminidase digestion and sodium periodate oxidation. The gradual decrease in CAMA3C8 binding upon periodate oxidation from 0.1Mm to 20Mm periodate concentrations suggests that the epitope defined by CAMA3C8 involves carbohydrates. Susceptibility to periodate is another hallmark of carbohydrate epitopes.

The antigen is susceptible to pronase and alkali borohydride treatment indicating that the carbohydrate moiety is linked to protein by glycosidic bonds. The same properties have been ascribed to the DF3 [13] and CAL antigens. The O-glycosidic linkage of carbohydrate to protein is between the hydroxyl groups of serine or threonine and the reducing ends of N-Acetyl galactosamines. The antigens typically contains a high proportion of O-Linked oligosaccharides, with an apparent molecular weight of 270,000 and because of their chemical similarities to glycoproteins found in mucus secretions, are generally referred to as mucin like.

Considering the frequent presence of sialic acid in antigenic determinants of breast carcinoma associated antigens as mucins recognized by MAbs DF3[13] B72.3 or CAL[14]. In this study affinity purified antigen has been treated with neuraminidase. In each case, however neuraminidase treatment of the mucin also significantly decreased antigenic activity, demonstrating that sialic acid is either a component of these epitopes or required for conformational integrity.

More than 25 derivatives of Neu NAC (N- acetyl neuraminic acid), have been reported to occur naturally, and these may be attached to a variety of residues by a number of linkages. The most common derivatives found in human`s is N-Acetylneuraminic acid (Neu 5 Ac) followed by the 5, 9-diacetyl, 7,9-diacetyl, 9-0 acetyl and 2-deoxy -2,3-didehydro derivatives. In glycol conjugates, sialic acids are α glycosidically linked to different positions of other sugars, most frequently to galactose or N-acetyl galactosamine and rarely to N-acetylglucosamine or sialic acid itself [20]. The reactivity of CAMA3C8 MAb was destroyed by neuraminidase treatment, since sialic acid is an essential part of the CAMA3C8 defined epitope.

In this study sialidase from Arthrobacter ureafaciens, was used which hydrolyzed α (2-3) glycosidic linkage much faster than α (2-6) bonds. Furthermore, the susceptibility of CAMA3C8 defined antigen to treatment with Arthrobacter ureafaciens neuraminidase which is specific for α (2-6) linked Neu NAC (indicate that the Neu NAc may be attached α (2-6) to peptide.

The highly glycosylated and polydisperse nature of this antigen, the results of various enzyme treatments and preliminary chemical composition data indicated that it is a mucin like antigen. CAMA3C8 defined antigen is a neuramindase sensitive carbohydrate epitope. However, the reactivity of MAb CAMA3C8 is also reduced by protease treatment of the mucin which suggests that CAMA3C8 bind to the sialylated carbohydrate in a protein conformation dependent manner. These results suggest that CAMA3C8 defined antigen is a Neu NAc α (2-6)- O→ Ser/Thr, which represents a unique tumor associated epitope.

CONCLUSION

We conclude that the CAMA3C8 recognizes an antigen which is highly glycated with O-linked carbohydrate moiety with N-Terminal neuraminic acid characteristic of polymorphic epithelial mucin.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

REFERENCES

1. Laboisse C, Bogomoletz Wv. (1989) Les micins:desglycoproteines enquete de reconnaissance. Ann.pthol. 9:175-180.

2. Roth J. (1984) Cytochemical localization of terminal – N- D galactosamine residues in cellular compartments of intestinal goblet cells: implication for the topology of O-glycosylation. J.Cell Biol. 98:399-405.

3. Goldenberg DM.(2002) Targeted therapy of cancer with radio labeled antibodies. J. Nucl. Med. 43:693-713.

4. Delong S, Davidson E. (1981) Preparation and properties of glycoprotein associated with malignancy. Biochemistry. 20:1047-1054.

5. Zacharius RM, Zell T.E, Morrision JH and Woodlock JJ. (1969) Glycoprotein staining following electrophoresis on acrylamide gels. Anal. Biochem. 30:148-155.

6. Kusama K, Hayasaka N, Moro I, Umemura S and Sekine T. (1986) A complementary histochemical method for a detection of a carbohydrate antigen. Jpn.J.oral Biol. 28:367-369.

7. Hsu SM, Raine L and Fanger H. (1981) Use of avidin – biotin-peroxidase complex (ABC) in immunoperoxidase techniques: comparison between ABC and unlabelled antibody (PAP) procedures. J. Histochem.Cytochem. 29:577-580.

8. K. Kothari, K. Bapat, A. Korde. (2204)Radiochemical and Biological Studies of Re Labeled Monoclonal Antibody- CAMA3C8 Specific for Breast Cancer," Indian Journal of Nuclear Medicine, vol. 19, no. 1, p. 11.

9. McManus JFA and Cason JE. (1950) Carbohydrate histochemistry studied by acetylation techniques I. periodic acid methods. J.Exp. Med. 91:651-655.

10. Woodward MP, Young W W and Bloodgood RA. (1985) Detection of monoclonal antibodies specific for carbohydrate epitope using periodate oxidation. J.immunol.Methods. 78:143-150.

11. Kjeldsen T, Clausen H and Hirohashi S.(1988) Preparation and characterization of monoclonal antibodies directed to the tumor-associated O-linked sialosyl-2-6α-Acetyl galactosaninyl (Siatosyl-Tn) epitope. Cancer Res. 48:2214-2220.

12. Licia Uccelli, Petra Martini, Micol Pasquali. (2017) Monoclonal Antibodies Radio labeling with Rhenium-188 for Radioimmunotherapy. BioMed Research International. 2017:1-7

13. Abe M and kufe D. (1989) Structural analysis of the DF3 human breast carcinoma Associated protein. Cancer Res. 49:2834-2839.

14. Hayes DF, Sekine H, Ohno T, Abe M, Kneefe K and Kerfe DW. (1985) Use of murine monoclonal antibody for detection of circulating plasma DF3 antigen levels in breast cancer patients. J.Clin.Invest. 75:1671-1678.

15. D. M. Goldenberg and R. M. Sharkey. (2011)Recent progress in cancer therapy with radiolabeled monoclonal antibodies," Therapeutic Delivery, vol. 2, no. 6, pp. 675–679.

16. Sekine H, Ohno T, Kerfe DW. (1985) Purification and characterization of a high molecular weight glycoprotein detectable in human milk and breast carcinoms. J.Immunol. 135:3610-3615.

17. Prat M, morra I, Bussolati G and Comoglio PM. (1985) C-AR3 a monoclonal antibody defined antigen expressed on human carcinomas. Cancer Res. 45:5799-5807.

18. Johnson VG, Schlom J, Paterson AJ. (1985) Analysis of a human tumor associated glycoprotein (TAG-72) identified by monoclonal antibody B72.3. Cancer Res. 46:850-857.

19. Gendler SJ, Taylor Papadimitriou, Duhig T, Rothbard J. (1990) Molecular cloning and expression of the human tumor associated polymorphic epithelial mucin, PEM. J.Biol.Chem. 266:12286-12290.

20. Schauer R. (1985) Sialic acids and their role as biological masks. Trends Biochem. Sci. 10:357-360.