

## Review Article

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# Human mammaglobin in breast cancer: a brief review of its clinical utility

Fawwaz Shakir Al Joudi

*Royal College of Medicine Perak, University of Kuala Lumpur, Perak Darul Ridzuan, Malaysia*

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**Human mammaglobin is a member of the uteroglobin proteins family that has recently been tested as a specific marker for breast cancer. While low levels may be seen in normal breast tissue, expression is increased dramatically in breast cancer and is correlated with higher grade. Detection in blood and body fluids is also correlated with cancer metastasis, and its levels with prognosis. This promises to be a useful screen for early detection of breast cancer, especially in high risk individuals. Mammaglobin has also been used for immunotherapeutic targeting of breast cancer cells. However, there are some controversies regarding its diagnostic efficacy and prognostic value, which warrant further study.**

**Key words** Breast cancer - diagnosis - immunotherapy - mammaglobin - prognosis

## Introduction

Despite advances in the biology of cancer, there remains a growing need for improved diagnostic accuracy<sup>1,2</sup>. In most tumours, there is a lack of tissue or serum molecular markers with sufficient sensitivity and/or specificity for detection, and disease evolution. For breast cancer, carcinoembryonic antigen (CEA) and CA 15-3 (CA 27-29) are common markers, although these lack sensitivity and specificity, are rarely elevated prior to gross disease, and are not seen in many patients with metastases<sup>3,4</sup>. Many other biomarkers have been suggested for use in detecting breast cancer in tissues, peripheral blood and/or bone marrow including human mammaglobin, cytokeratins (CK19 & CK20), survivin, polymorphic epithelial mucin (MUC-1), epidermal growth factor receptor (EGFR), maspin,

estrogen receptor (ER) and progesterone receptor (PR), urokinase plasminogen activator (uPA), plasminogen activator inhibitor-1 (PAI-1) and B726P and small breast epithelial mucin (SBEM)<sup>5-9</sup>. Most, however, are neither sufficiently sensitive, nor tumour-specific, to be clinically useful<sup>10</sup>. Clinically, ER, PR and human epidermal growth factor receptor 2 (HER-2) are the most useful markers for prognosis and therapy but not for diagnosis or evolution<sup>8,11,12</sup>. The search for new markers in breast cancer is still ongoing. This review attempts to evaluate human mammaglobin as a promising diagnostic, prognostic and potentially therapeutic tool in breast cancer.

## Discovery and nomenclature of human mammaglobin

In 1994, Watson and Fleming recognized sequence tags expressed in neoplastic mammary epithelial tissue

by using a modified differential display polymerase chain reaction (PCR) technique which was developed to detect and compare mRNA differential expression among cells<sup>13</sup>. One of these sequence tags led to the discovery and isolation of a novel full length cDNA that encoded a protein, now known as the human mammaglobin (hMAG)<sup>10</sup>. Human mammaglobins A and B are homologues and members of a large family, and subsequently have been repeatedly reported as potentially valuable in breast cancer diagnosis and prognosis<sup>14-18</sup>. Nevertheless, hMAG-B subtypes (hMAG B-1 and B-2) have also been detected in endometrioid endometrial carcinoma especially in well- and moderately-differentiated tumours<sup>19-23</sup>, and hMAG A was also detected in a case of a poorly differentiated myoepithelial cell rich carcinoma, though this may have been of breast origin<sup>24</sup>. The nomenclature used in the literature shows various other names and abbreviations for mammaglobin variants and their genes, such as MAM, MGB, UGB3, MMG, SCGB2A1. In this review, the abbreviations quoted will be restricted to hMAG-A and hMAG-B unless otherwise stated.

### **Chemistry and regulation of hMAG**

Human MAG was described as one of the 23 members of the uteroglobin/Clara cell protein family of small epithelial secretory proteins, the secretoglobins<sup>25-27</sup>. It is a 93-amino acid protein with two N-linked glycosylation sites, both linked to approximately 3 kDa carbohydrate chains, with molecular masses of 23.4 and 16.2 kDa for the glycosylated and deglycosylated complexes, respectively<sup>27</sup>. Mammaglobin forms soluble, covalent heterodimers with lipophilin B in an anti-parallel manner that allows the formation of three disulphide bridges between the two molecules<sup>28,29</sup>. The mammaglobin protein forms four alpha helices that sandwich in a head-to-tail orientation creating a hydrophobic core, allowing the formation of the three closely situated disulfide bonds. In this model, an N-terminal cysteine of mammaglobin is covalently linked to the C-terminal cysteine of lipophilin B. The protein serological marker in breast cancer appears to reside in the sequences and framework of the hMAG-lipophilin complex<sup>28</sup>. The multigene family of MAG A and MAG B is localized on chromosome 11q12.2 in a dense cluster spanning not more than 400 kbp. In this same gene locus, the human Clara Cell 10-kDa protein (CC10) gene, mammaglobins A and B, lipophilins A and B, lacryglobin, and another member called lymphoglobin, are included<sup>26,30</sup>.

By Western blotting analysis, the MAG-A protein was found to exist in the mammary tissue in two main forms with approximate molecular masses of 18 and 25 kDa. Both forms were detected more frequently in breast carcinomas than in fibroadenomas or in normal breast tissues. Furthermore, an inverse relationship was found between the high molecular weight form of MAG and both tumour grade and proliferative index. No significant correlation was found between the MAG proteins and either tumour size or nodal status, although it was associated with a favourable prognosis for breast cancer<sup>31</sup>.

Although the secretoglobins are known to be regulated by steroid hormones, mammaglobin expression is not induced by estrogens in ER-positive breast cancer cell lines, such as MCF7 and T47D implying that mammaglobin is almost exclusively expressed in the mammary gland independent of steroid hormones<sup>32</sup>. Thus, PEA-3 expression vector and some repetitive sequences may be involved in the regulation of hMAG expression<sup>33</sup>. In breast tumours, hMAG A and lipophilin appear to be co-expressed simultaneously, suggesting the existence of common regulatory mechanisms<sup>16</sup>.

### **Function of mammaglobin protein**

The function of mammaglobin protein is not known. Northern blot and RT-PCR analyses have demonstrated that hMAG expression is restricted to the mammary gland and that hMAG mRNA is highly detectable in human breast tumour cell lines and primary breast tumours compared to non malignant breast tissue<sup>27,32</sup>. However, its expression did not increase the growth rate of cell lines, indicating that it is not likely to be involved in a cell division function<sup>34</sup>. It was also expected to be readily secreted from breast tumours and to elicit production of autoantibodies detectable in the serum of breast cancer patients and women at high risk of breast cancer<sup>35</sup>, raising the question of whether this may be used as a marker. Moreover, the hydrophobic core is capable of binding steroid-like molecules, suggesting the existence of a hormonal transport or activation function<sup>28,36</sup>.

### **Mammaglobin in breast cancer**

In preliminary attempts at determining the clinical utility of mammaglobin as a breast tumour marker, Watson and Fleming<sup>27</sup> found that mammaglobin mRNA expression was multiplied by at least 10-fold when compared to normal breast tissue, using RT-PCR and Northern blot analysis, whereas by

immunohistochemistry, they detected hMAG in 91 per cent of the breast cancer cases, independent of stage and histological type<sup>35</sup>. Similarly, a number of subsequent studies described the detection of mammaglobin at high levels in primary breast cancers, while it was either undetectable in non-breast tumours or present at low levels in healthy breast tissue, but not in other tissues, making it a suitable candidate for diagnosis of breast cancer<sup>37-44</sup>. Human MAG was also used in differentiating between the different sub-types of breast carcinomas, such as luminal A and B, HER-2, basal-like carcinoma (BLC), and unclassified triple-negative carcinoma (UTNC) by established surrogate immunohistochemical profiles<sup>45</sup>. Furthermore, MAG-A positive expression by IHC staining was found in approximately 90 per cent of invasive ductal carcinoma and in 80 per cent of intraductal carcinoma<sup>46</sup>. In addition, the over-expression of the hMAG gene was correlated with high grade breast cancer<sup>37,47</sup>. Hence, the usefulness of hMAG in diagnosis stems from its abundant detection in breast tumours, the low existence in tissues and tumours other than breast, and its efficiency in detecting residual disease and predicting recurrence<sup>6</sup>. However, controversies have been reported as the correlation between blood hMAG and grading has fluctuated<sup>31,48</sup>, and hMAG was detected, by tissue microarray, in 44 of the 544 non-breast tumours<sup>49</sup>.

#### **MAG in metastatic lesions and in the blood and fluids of breast cancer patients**

The detection of hMAG has been reported, both in the blood and stem cells of about 60 per cent of patients with breast cancer<sup>50,51</sup>. However, only 11 per cent detection was reported by the Suchi group<sup>52</sup>. Using the nested reverse transcriptase polymerase chain reaction (RT-PCR) assay, hMAG was detected by the Zach group in the blood of about quarter of 114 breast cancer patients, and in about half (35/ 81) of those with metastatic disease, but in none of healthy controls<sup>53</sup>. The same group investigated hMAG expression in cases of suspected breast cancer and found it positive in 43 per cent of 81 patients with metastatic breast cancer, but in less than 3 per cent in other test groups<sup>54</sup>. The general implication from these studies is that hMAG can be a reliable indicator or detector of metastasis. In cases of occult tumours of unknown origins, it was also found that detection of hMAG mRNA may aid in detecting metastatic breast tumours and in detecting breast cancer micrometastasis<sup>55-61</sup>.

The detection of hMAG-B was reported to be more evident during the later stages of cancer whereas

maspin was detectable during earlier stages<sup>62</sup>. However, the detection of maspin can be influenced by a number of cytokines whereas hMAG is not<sup>63</sup>. hMAG detection by RT-PCR may be very useful in detecting occult cells in breast cancer patients<sup>64</sup>.

Hence, mammaglobin may be an important tool for detecting primary or metastatic breast cancer, monitoring lymph nodes during and after surgery, and predicting disease outcomes and recurrences<sup>65</sup>. hMAG elevation was associated with distant recurrences and decreased survival periods, and has been of high specificity for lymph nodal involvement<sup>66,67</sup>. Furthermore, it was also elevated in brain metastases in breast cancer patients<sup>68,69</sup>, used in the diagnosis and detection of metastases in breast cancer patients with pleural effusions or other metastases and recurrences<sup>70-75</sup>. In addition, it could also help differentiate between cutaneous apocrine carcinoma and breast metastases<sup>76</sup>, and in distinguishing metastatic breast carcinoma from a primary ovarian or uterine malignancy<sup>77</sup> or abdominal cancers<sup>78</sup>. In detecting minimum residual disease in the bone marrow of breast cancer patients, MAG-A, along with maspin, was also useful<sup>6,59,79</sup>. The use of fluorescence *in situ* hybridization (FISH) along with RT-PCR, can improve the detection of metastases, contribute to the decision on tumour staging, assess treatment efficiency and indicate prognoses<sup>80</sup>.

Altogether hMAG-A detection in breast cancer is of high specificity, but its sensitivity is low and can be enhanced by the simultaneous detection of Ki-67<sup>81</sup>. Generally, the expression of MAG-A along with B726P, small breast epithelial mucin (SBEM) and MUC1 was useful in differentiating breast tissues from non-breast tissues<sup>24</sup>. Although efficiency of the RT-PCR used can improve the detection<sup>82</sup>, critical views and findings have been reported with the implication that hMAG-A has only a limited value in identifying the mammary origin of metastatic carcinomas<sup>83</sup>. To further understand the significance of hMAG, a direct comparison of the clinical validity of hMAG with other markers, was done and summarized in the Table. hMAG mRNA appears to be more stable and detectable in the blood for relatively longer periods than other breast cancer markers, making it useful where samples are stored for short periods<sup>88</sup>.

#### **Mammaglobin detection in lymph nodes**

Although MAG and CEA were detected in all breast cancer cell lines tested, these were not detected in normal lymph nodes<sup>87</sup>. Similarly mammaglobin was

**Table.** A comparison of the efficiencies of hMAG with various other breast cancer markers as reported in the literature

Tumour marker	Specific remarks	References
Carcinoembryonic antigen (CEA)	Detected in recurrence and associated with circulating tumour cells detection, but is of low sensitivity.	6, 8, 96
Cytokeratins (CK19 and CK20)	Low sensitivity and are expressed in normal cells and other tumours.	6
Epidermal growth factor receptor (EGFR)	Low sensitivity and are expressed in normal cells and other tumours.	6
Maspin	May protect against recurrence.	85
Polymorphic epithelial mucin (MUC-1)	High pre-operative levels of CA 15-3 associate with adverse outcomes. CA27.29 is of little value. These are also of low sensitivity and are expressed in normal cells and haematological tumours. Levels of detection 42% in breast cancer, but approximately 59% in non-breast tumours.	3, 6, 24
B726P	In conjunction with hMAG, it may help differentiate mammary from non-mammary tissue.	9
Urokinase plasminogen activator (uPA)	May aid in assessing prognosis.	4, 8
Plasminogen activator inhibitor 1 (PAI-1)	May aid in assessing prognosis.	8
Oestrogen receptor (ER)	May be detected in primary lung adenocarcinomas. In breast cancer, it is used in predicting response to hormonal therapy. It has a limited prognostic value.	12, 86
Progesterone receptor (PR)	Can be the basis for hormonal therapy.	4
Human epidermal growth factor receptor-2(HER-2)	Low expression in cancer cells, though it helps in selecting patients for herceptin therapy.	8
Breast cancer 1 and 2 early onset (BRAC-1 and BRAC-2)	Can be used to detect high risk patients.	4
Small breast epithelial mucin (SBEM)	Detected in only 52% of breast tumours. None in non-breast tumours.	9
Survivin	Not specific for breast cancer.	5
Ki67	May serve as an index for breast cancer progression.	87
Human mammaglobin (hMAG)	80-90% expression in breast tumours, with a 97% sensitivity in detecting residual disease	6

detected in 100 per cent of histologically positive breast cancer lymph node samples, whereas no mammaglobin expression was observed in lymph nodes and in sentinel lymph nodes from cases without histologically detectable breast tumour cells<sup>40,64,66,89-92</sup>. In addition, the experimental set-up of conjugating the near infra red (NIR) fluorescent dye VivoTag-S 680 to a murine monoclonal antibody against human mammaglobin-A was a promising additive as an imaging probe for the non-invasive detection of breast tumour metastasis in lymph nodes in mice<sup>46</sup>. The detection of circulating tumour cells (CTCs) was found to be enhanced by using a combination of markers along with hMAG such as cytokeratin 19, and this combination can be useful clinically for intra- or post-operative axillary lymph node dissection decisions in breast lymph nodes<sup>93-95</sup>, although cytokeratin 19 was found to be more specific than hMAG, when probed by RT-PCR in lymph nodal detection of breast cancer<sup>96</sup>. However, RT-PCR detection of hMAG was described as being

more informative than both cytokeratin 19 and CEA regarding the molecular detection in sentinel lymph nodes<sup>97</sup>.

#### **Mammaglobin correlation with other breast cancer markers**

The analysis of over 300 tumours revealed that hMAG-A and lipophilin are co-expressed simultaneously<sup>16</sup>. Although mammaglobin is more specific and sensitive than epidermal growth factor receptor (EGF-R) and cytokeratin-19<sup>98</sup>, when tested with CEA and CA-15.3 simultaneously, its sensitivity was increased from 54 per cent to approximately 90 per cent, suggesting the usefulness of mammaglobin mRNA as an adjunct to these markers<sup>84</sup>. Samples from breast cancer patients, patients with benign breast tumours, healthy individuals, and patients with other solid tumours, investigated by quantitative RT-PCR for hMAG, survivin and human telomerase reverse transcriptase (hTERT) have shown that the three markers



together, make up a powerful tool for detecting CTCs of breast cancer, with very high specificity, and that these were correlated with lymph nodal involvement. hMAG mRNA was specific for breast cancer, whereas survivin and hTERT were detected in the serum samples of patients with other solid tumours<sup>99</sup>. Other groups investigated parallel testing of the markers CEA mRNA, CK-19, HER-2, MAG-A, MAGEA3 (melanoma antigen family A, 3), TWIST homolog 1(Drosophila) [TWIST-1) and hydroxymethylbilane synthase (HMBS). This appeared to increase the sensitivity for detecting CTCs with higher detection rates found in cases with metastasis<sup>100</sup>. Controversial results appeared regarding the correlation of hMAG expression with ER positive and negative tumours<sup>53,101,102</sup>. Correlations of hMAG with Ki67, ER status and other clinicopathological factors have been found to increase the detection and diagnostic efficiency, and it was also concluded that hMAG correlated, for unknown reasons, with the G3 histologic grading of breast cancer<sup>43,82</sup>. There were also associations between MAG expression and gross cystic disease fluid protein 15 (GCDFP-15) and with the molecular sub-types of breast carcinoma, such as basal-like carcinoma (BLC), and unclassified triple-negative carcinoma (UTNC). These same markers can be useful in detecting recurrences and distant metastases of breast tumours including detecting these in pleural effusions<sup>45,71,76,103</sup>. Similarly, a combination of markers including mammaglobin detected by immunohistochemistry (IHC) stains raised the sensitivity and specificity in distinguishing between cutaneous metastases of breast carcinomas (CMBCs) and sweat gland carcinomas (SGCs)<sup>104</sup>. In conclusion, in addition to the efficiency of hMAG in diagnosis and in detecting micrometastasis, its efficiency can be magnified by combining it with other markers.

### **Mammaglobin in assessing prognosis of breast cancer**

Tumour size, extent of axillary node involvement, high histologic grade, bone marrow involvement, and the detection of CTCs are considered strong predictors of breast cancer recurrence, and of poor prognosis for patients with metastatic tumours<sup>105-106</sup>. Disseminated tumour cells in the bone marrow or in the blood, assessed by TWIST1, CK19 and MAG A mRNA, in addition to trefoil factor 1 (TFF-1) were correlated with short recurrence periods and poor outcomes<sup>67,107-108</sup>. Furthermore, hMAG detection in CTCs of the peripheral blood was a poor prognostic indicator<sup>47,102,109</sup>. Poor prognosis was also reported to be associated

with detection of hMAG in leukapheresis products in individuals with high risk for breast cancer<sup>110</sup>. There are, however, controversies based on experimental findings in which either no correlation was established between hMAG detection in the blood of breast cancer patients and outcomes or other known prognostic factors<sup>111</sup>, or that mammaglobin was described as being a good prognostic indicator as well as being a useful indicator of metastases<sup>41</sup>, and the detection of hMAG mRNA in the blood of breast cancer patients did not correlate with the development of recurrences<sup>112</sup>. In addition, the expression of hMAG has been reported to be correlated with less aggressive mammary tumours<sup>113</sup>. Moreover, *in vitro* observations showed that over-expression of hMAG did not have a great effect on the growth rate of the breast carcinoma cell line Hs578T<sup>34</sup>. Hence, the views on the relationship between hMAG and prognosis are highly controversial.

The controversies reported may have been attributed to the ethnic differences as well as technical differences such as the blood volume used, in addition to the sensitivity of the assays used especially regarding the selection of primers and probes as well as the individual difference among patients. Moreover, immunohistochemical detection might have been accompanied by some bias in interpretation in different laboratories using kits and antibodies of different antigen detecting specificities and of dissimilar affinities.

### **Human MAG and cancer immunotherapy**

In addition to its diagnostic role, hMAG also provided a new tool for the prognosis and management of breast cancer patients<sup>35,51</sup>. It was also concluded that the efficiency of therapy may be assessed by measuring the levels of hMAG mRNA by RT-PCR<sup>114</sup>. Also, a higher percentage of positive mammaglobin cases has been found among patients with metastatic and progressive breast cancer than in those responding to therapy<sup>115</sup>, as this may aid in detecting disseminated breast cancer and in making decisions for therapy<sup>69,116</sup>.

Human MAG may also enhance the possibility of establishing effective targeted therapies or vaccines against breast cancer cells through targeting mammaglobin-derived epitopes on cytotoxic T-lymphocytes<sup>117-119</sup>, for effective delivery of targeted therapies using a transmembrane N-terminal domain of hMAG<sup>120</sup> or MAG-A-derived epitopes with HLA-A-2, HLA-A-3, and HLA-A-24, CD8 restricted responses<sup>121-125</sup>. Being a membrane-associated protein,

hMAG may serve as a molecular marker for effective targeted drug therapies for breast cancer<sup>120</sup>. It may also aid in the preparation of experimental vaccines directed against breast cancer cells by introducing MAG-A DNA vaccine<sup>117</sup>, or by stimulating CD4(+) CD25(-) T cells *in vitro* with MAG-A-pulsed antigen-presenting cells<sup>124</sup> or by transduction of dendritic cells with a Tat-MAG<sup>127</sup>. The notion that MAG can bind breast cancer cells has brought up speculations about its possible usefulness in directing radioisotopes or toxins to target these cells<sup>128</sup>. Another suggested therapeutic approach has been the use of the MAG promoter to deliver, by gene therapy, oncolytic viruses or toxic genes to mammary tumours<sup>129</sup>. Moreover, a combination of targets such as hMAG combined with HER-2/neu in active immunotherapy may enhance the therapeutic vaccine efficiency and specific T cells for use in adoptive immunotherapy for the treatment of established metastatic disease<sup>130</sup>. Nevertheless, a recent report stated that upregulation to induce over-expression of h-MAG in breast cancer cells can reduce the metastatic potential of these cells<sup>131</sup>. This approach may prove to have an additive value in the management of aggressive breast cancer.

### Mammaglobin is a promising breast marker

Mammaglobin, known for its mammary tissue specificity, has been considered a promising diagnostic marker in breast cancer for almost 10 years. In particular, the application of mammaglobin RT-PCR to detect disseminated breast cancer cells has been reported. Much work has evaluated the detection of mammaglobin mRNA in lymph nodes, blood, and bone marrow of breast cancer patients. Structural details about the mammaglobin complex have also been discovered, and these findings can be implemented to optimize detection of the secreted protein<sup>18</sup>. The peculiarity of hMAG lies in its almost sole existence in mammary tissue and mammary carcinoma. In addition, the heightened expression in carcinomas and its association with tumour grades renders it an excellent marker for diagnosis and prognosis. Methods are being studied to screen and detect early breast cancer. To date, only mammography has been the method of early detection and hMAG may enhance and complement the predictive value of mammography<sup>9</sup>.

### Conclusions

Looking at the various reports and opinions laid down in the literature, human MAG does carry the potential of being a molecule of diagnostic, prognostic,

as well as therapeutic significance. Furthermore, it appears to have an additional potential usefulness as a screening tool for breast cancer. More studies will have to be analysed to explain the differences of results found by some groups.

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*Reprint requests:* Prof. Dr Fawwaz Shakir Al Joudi, Royal College of Medicine Perak, Universiti Kuala Lumpur, No. 3 Jalan Greentown, 30450 Ipoh, Perak Darul Ridzuan, Malaysia  
e-mail: fajoudi@yahoo.com