85

GLYCOSYLATED OLEIC ACID/VITAMIN D-BINDING PROTEIN SUPPRESSES HER2 ONCOGENE EXPRESSION IN HUMAN BREAST CANCER

Marco Ruggiero[1,3], Jacopo J.V. Branca[2], David Noakes[3], Massimo Gulisano[2], Gabriele Morucci[2], Lynda Thyer[3] and Stefania Pacini[2]

Departments of [1]Experimental and Clinical Biomedical Sciences and [2]Experimental and Clinical Medicine University of Firenze, Firenze, Italy;
[3]Immuno Biotech Ltd; Guernsey, Channel Islands, UK

A woman was diagnosed with mammary adenocarcinoma in the right breast in 1985 at the age of 37, followed by quadrantectomy, lymphadenectomy and irradiation. In 1999, an adenocarcinoma was diagnosed in the left breast, followed by ample resection and anti-oestrogen receptor treatment for 6 years. In April 2014, an infiltrating adenocarcinoma was diagnosed in the right breast that had been operated in 1985. Pre-operative biopsy showed weak positivity for progesterone receptor (PgR, <1%) and high positivity for the oncogene HER2 (>10%, score 2+). With the goal of boosting her immune system during the 3 weeks preceding surgery, glycosylated oleic acid/vitamin D-binding protein (OA-GcMAF) was administered by subcutaneous injections, nebulisation and with a fermented milk product rich in OA-GcMAF. No drug was administered in the 3 weeks preceding surgery, nor had the patient received any treatment for the previous 8 years. Following right mastectomy, analysis of the surgical specimen showed no positivity for HER2 expression (negative, score 0) and significant increase in positivity of PgR, from <1% to 20%. These results indicate that OA-GcMAF treatment suppressed oncogene expression and induced differentiation of cancer cells. *Introduction:* The healthy properties of oleic acid (OA) in breast cancer have been known for centuries (1) and recent evidences suggest that these properties are amplified by association of OA with proteins such as α-lactalbumin and lactoferrins. These proteins form OA-protein complexes that exhibit highly selective anti-tumour activity *in vitro* and *in vivo* (2). We recently demonstrated that also a serum protein with the capability to bind OA shows anticancer effects; this is the glycosylated vitamin D-binding protein also known as Gc-protein-derived macrophage activating factor or GcMAF (3). This protein binds both OA and vitamin D and exerts its immune-stimulating and anticancer effects through cross-talk with the vitamin D receptor (4). Here we report a clinical observation suggesting that OA-GcMAF, that is GcMAF-complexed with OA, suppresses the expression of a major oncogene involved in human breast cancer that is the human epidermal growth factor receptor 2 (HER2). *Patients and Methods:* A woman was diagnosed with mammary adenocarcinoma in the right breast in 1985 at the age of 37, followed by quadrantectomy, lymphadenectomy and irradiation. In 1999, an adenocarcinoma was diagnosed in the left breast, followed by ample resection and anti-oestrogen receptor treatment for 6 years. In April 2014, an infiltrating adenocarcinoma was diagnosed in the right breast that had been operated in 1985. With the goal of boosting her immune system during the 3 weeks elapsing between biopsy and programmed surgery, OA-GcMAF (Goleic®, Immuno Biotech Ltd.) was administered by subcutaneous injections (880 ng) and nebulisation (880 ng) as indicated in (3). The patient followed a nutritional regime based on a low carbohydrate, high protein diet (5). To this end, the patient was provided with food containing only 2% carbohydrates (Le Gamberi Foods, Forlì, Italy), and with essential aminoacids (Master Aminoacid Pattern®, dr. reinwald healthcare gmbh, Schwarzenbruck, Germany) (6). The patient was also provided with a fermented milk product containing colostrum and microorganisms known to produce OA-GcMAF from the Gc-protein present in milk (Bravo Probiotic®, Les Alpes, Wellington, NZ). No drug was administered or was programmed in the 3 weeks preceding surgery, nor had the patient received any treatment for the previous 8 years. The analyses on HER2 and other gene expression on the biopsy and surgical specimens were performed by the laboratory of the University Hospital of Careggi of the Italian Public Health Service, in Firenze, Italy. Analyses were performed according to the European standards of quality (UNI EN ISO 9001:2008) and were examined and countersigned by four different professionals. The original documents are conserved in the archives of the Department of Biomedicine of the Careggi

Hospital (Molecular Diagnostic and Pathologic Histology). The patient gave the informed consent to the treatment as well as to this description of her results. Surgery was performed at the Division of General Surgery n. 2 of the University Hospital of Careggi. *Results:* Amplification or overexpression of HER2 plays an important role in the development and progression of breast cancer and has become an important biomarker and target of therapy (7) since it is strongly associated with increased disease recurrence and a poor prognosis (8). Consistent with the aggressive nature of the cancer in this patient, pre-operative biopsy on four specimens collected under ultrasound guidance showed significant positivity for HER2 assessed by the polyclonal antibody A 0485 with >10% of positivity and a score of 2+ (Figure 1). After 3 weeks of OA-GcMAF treatment and subsequent mastectomy, analysis of the surgical specimen showed no positivity for HER2 expression (negative, score 0; Figure 1), thus indicating complete suppression of oncogene expression. Study of the expression of progesterone receptor (PgR, clone 1E2) was consistent with such a reversal of the neoplastic phenotype. PgR expression in the biopsy was low (<1%), a finding consistent with poor differentiation and aggressiveness. However, in the surgical specimen taken after the 3 weeks of treatment with OA-GcMAF, PgR expression was significantly increased to 20% (Figure). The selectivity of these effects was confirmed by a study of the expression of Ki67 and estrogen receptor (30% and 90%, respectively) that did not show any change following OA-GcMAF treatment (Figure). *Discussion:* These results demonstrate that OA-GcMAF, administered by subcutaneous injections, aerosol or in a functional food product, suppressed the expression of HER2, an oncogene which plays a key role in the aetiology, invasive progression and metastasis in breast cancer. This effect was paralleled by increase of PgR expression, thus indicating that OA-GcMAF treatment induced healthy differentiation of cancer cells. We hypothesize that these multifaceted effects on the regulation of gene expression in human breast cancer are due to the peculiar association of OA with GcMAF that is an association between two molecules endowed with anticancer properties. In fact, OA has been shown to down-regulate HER2 expression in cancer cell lines (9) and we demonstrated that GcMAF inhibits human breast cancer cell proliferation and reverts their malignant phenotype (10). We hypothesize that OA-GcMAF, but not OA or GcMAF taken singularly, interacts with the HER2 protein through hydrophobic interaction between the amino-terminal of GcMAF and the extracellular region of HER2, and between the OA-binding region of GcMAF and the plasma membrane (4). In fact, in the stretch of aminoacids between position 17-46 of GcMAF, and position 243-273 of HER2, there is a high density of hydrophobic aminoacids that may favour selective binding. Whatever the case, these results indicate that the effects of OA-GcMAF in cancer are due to a multiplicity of actions that involve suppression of oncogene expression.

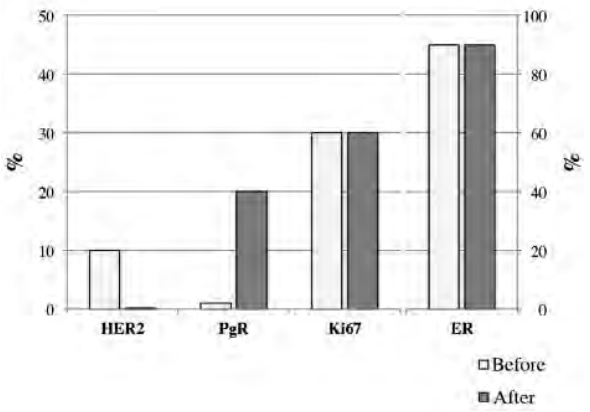

Figure. *Level of expression of HER2, progesterone receptor (PgR), Ki67 protein and estrogen receptor (ER).*

"Before", indicates level of expression observed on the biopsy samples obtained 3 weeks before surgery and before any treatment. "After", indicates level of expression observed on surgical specimens after OA-GcMAF treatment. Level of expression is expressed as percentages as in the original reports. The axis on the left (0-50%) refers to HER2, PgR and Ki67. The axis on the right (0-100%) refers only to ER. The actual levels were: HER2, before: >10%, score 2. HER2, after: negative, score 0. PgR, before: <1%. PgR, after: 20%. Ki67 before and after: 30%. ER, before and after: 90%.

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