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BRIEF COMMUNICATION

In Vivo Uptake of Rare Earth Metals by Triple-Negative Breast Cancer Cells

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Abstract Rare earth metals (REM) are a group of 17 chemical elements in the periodic table, namely scandium (Sc), yttrium (Y) and the lanthanides. In relation to atomic volume and geological behavior, the lanthanides are further subdivided into light, medium and heavy REM. They find many applications in the technological field; however, their impact on the human health is still conflicting and, for many aspects, unknown. During a research program carried on 113 cases of female breast cancer, immunohistochemically categorized in Her2-positive (29 cases), Her2-negative (57 cases) and triple negative (27 cases), aimed to evaluate the role of environmental particulate in carcinogenesis by elemental microanalysis, for the first time in literature we have detected a REM uptake, in detail europium (Eu), dysprosium (Dy) and praseodymium (Pr), inside the neoplastic cells belonging to a single triple negative breast cancer. Curiously, the woman affected by this form of malignancy had worked in the ceramic industry, a well-known source of REM, during her life, and she was the one and only patient of our series to be dedicated to this activity. The medical repercussions of our findings are here discussed: in fact, a REM detection in only 1 of 113 examined cases seems to exclude active roles in breast carcinogenesis and discloses new possibilities for therapeutic developments in triple negative breast cancer.

Keywords Triple-negative breast cancer · Rare earth metals (REM) · Europium (Eu) · Dysprosium (Dy) · Praseodymium (Pr) · Elemental microanalysis

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Introduction

As defined by the International Union of Pure and Applied Chemistry (IUPAC), rare earth metals (REM) are a group of 17 chemical elements in the periodic table, namely scandium (Sc), yttrium (Y) and the lanthanides [1]. The term 'rare earths' derives from the uncommon mineral gadolinite, also known ytterbite from the Sweden village Ytterby, where they were isolated for the first time in 1787 by Carl Axel Arrhenius [2]. Despite their name, REM can be found at high concentrations in the Earth's crust, with the exception of the promethium, which is very unstable. In relation to atomic volume and geological behavior, the lanthanides are further subdivided into:

- light REM: lanthanum (La), cerium (Ce), praseodymium (Pr), neodymium (Nd), promethium (Pm);
- medium REM: samarium (Sm), europium (Eu), gadolinium (Gd), terbium (Tb), dysprosium (Dy), holmium (Ho);
- heavy REM: erbium (Er), thulium (Tm), ytterbium (Yb), lutetium (Lu).

The REM find many applications in the technological field, for example they are used in the production of superconductors, magnets, catalysts, optical fibers and microwave resonators [3]. However, their impact on the human health is still conflicting and, for many aspects, unknown [4–6]. During a research program carried on 113 cases of female breast cancer, aimed to

evaluate the role of environmental particulate in carcinogenesis, for the first time in literature we have detected a REM uptake in the neoplastic cells belonging to a single woman, who curiously worked in the ceramic industry throughout her life. Here, we discuss the medical repercussions of our surprisingly finding, correlating them with the hormone receptor and Her2 status, the main prognostic factors for breast cancer [7].

Materials and Methods

Our research group has retrospectively investigated the presence of environmental particulate in 113 cases from as many female patients affected by breast ductal carcinoma, aged between 39 and 78 years old at the time of diagnosis. The specimens were fixed in 10% neutral buffered formalin, paraffin embedded and, then, submitted to routinely haematoxylin / eosin (H&E) staining and immunohistochemical characterization. After deparaffinization, hydration, endogenous peroxidase blocking and heat-induced antigen retrieval, the tissue sections were incubated for 30 min at room temperature with anti-E Cadherin (clone 36B5, prediluted; Ventana, Tucson, AZ, USA), anti-Ki67 (clone MIB-1, 1:75; Dako, Glostrup, Denmark), anti-estrogen receptor (ER, clone SP1, prediluted; Ventana), anti-progesterone receptor (PR, clone 1E2, prediluted; Ventana) and anti-Her2 (HercepTest; Dako). Biotinylated secondary antibody was applied and the staining product detected with avidin-biotin complex against a hematoxylin counterstain. Detection of the staining reaction was achieved by an enzyme conjugated polymer complex adapted for automatic stainers from Roche Ventana Medical Systems, with 3–3′ diaminobenzidine tetrahydrochloride (DAB) as chromogen. In order to evaluate the presence of environmental particulate inside the neoplastic cells, 20-μm-thick sections were prepared from representative formalin-fixed and paraffin-embedded blocks and used for elemental microanalysis according to Gatti and Montanari [8]. These paraffin sections were deposited on acetate sheets, deparaffined with xylol and mounted on aluminium stubs. Thereafter, they were inserted in the chamber of a field emission gun - environmental scanning electron microscope (ESEM Quanta200, FEI Company, Eindhoven, Netherlands) equipped with an energy dispersive system (X-EDS INCA-350, Oxford Instrument GmbH, Wiesbaden, Germany), in order to obtain the chemico-elemental characterization of the particulate matter in the neoplastic tissue.

Results

On H&E slides, all the cases have shown a main infiltrating carcinomatous component, intermingled with scattered foci of

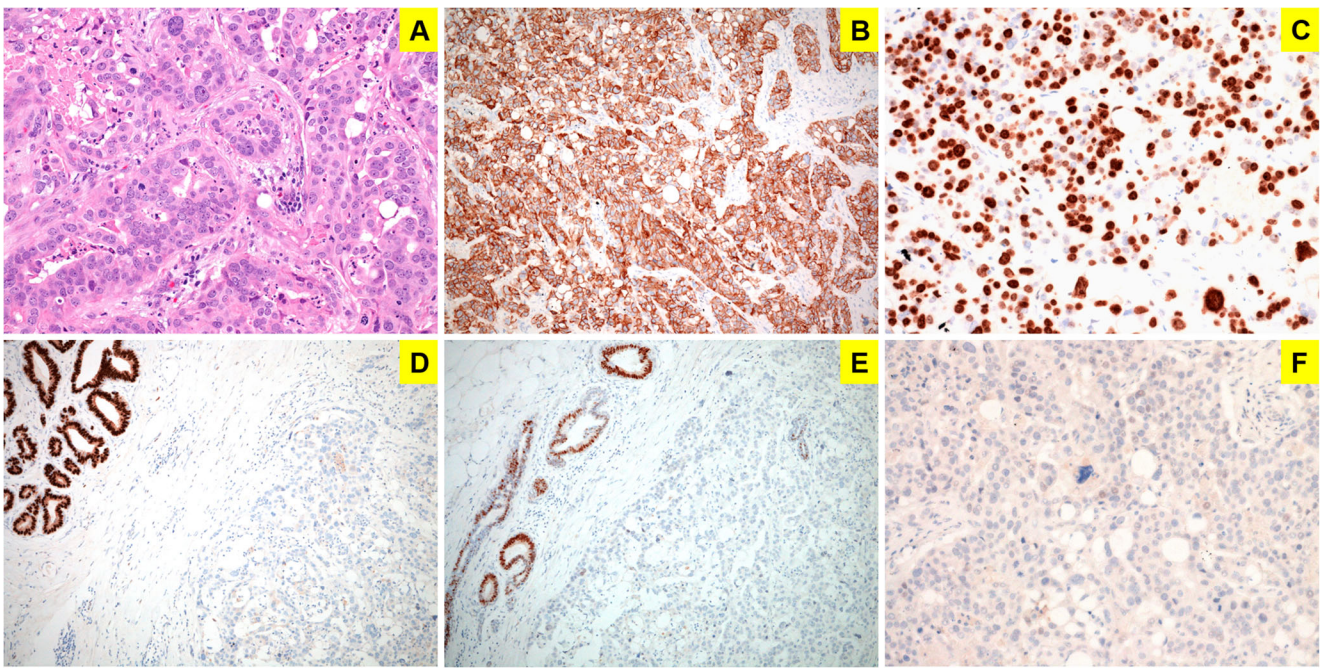

Fig. 1 The no special type (NST) invasive ductal carcinoma, which has shown an intracellular accumulation of REM, is here illustrated (**a**, H&E, 20×). It is a high grade malignancy (third grade according to Elston and Ellis criteria), E-Cadherin positive (**b**, prediluted clone 36B5, Ventana; 10×), with many areas of tumor necrosis and an elevated cytoproliferative index (**c**, 1:75 clone MIB-1, Dako; 10×); it does not exprime the receptors for estrogen (**d**, prediluted clone SP1, Ventana; 4×) and progesteron (**e**, prediluted clone 1E2, Ventana; 4×), both well preserved in the residual ducts (**d** and **e**) and it shows no reactivity for Her2 test (**f**, HercepTest, Dako; 20×). For these reasons, it can be labeled as triple negative breast cancer (TNBC)

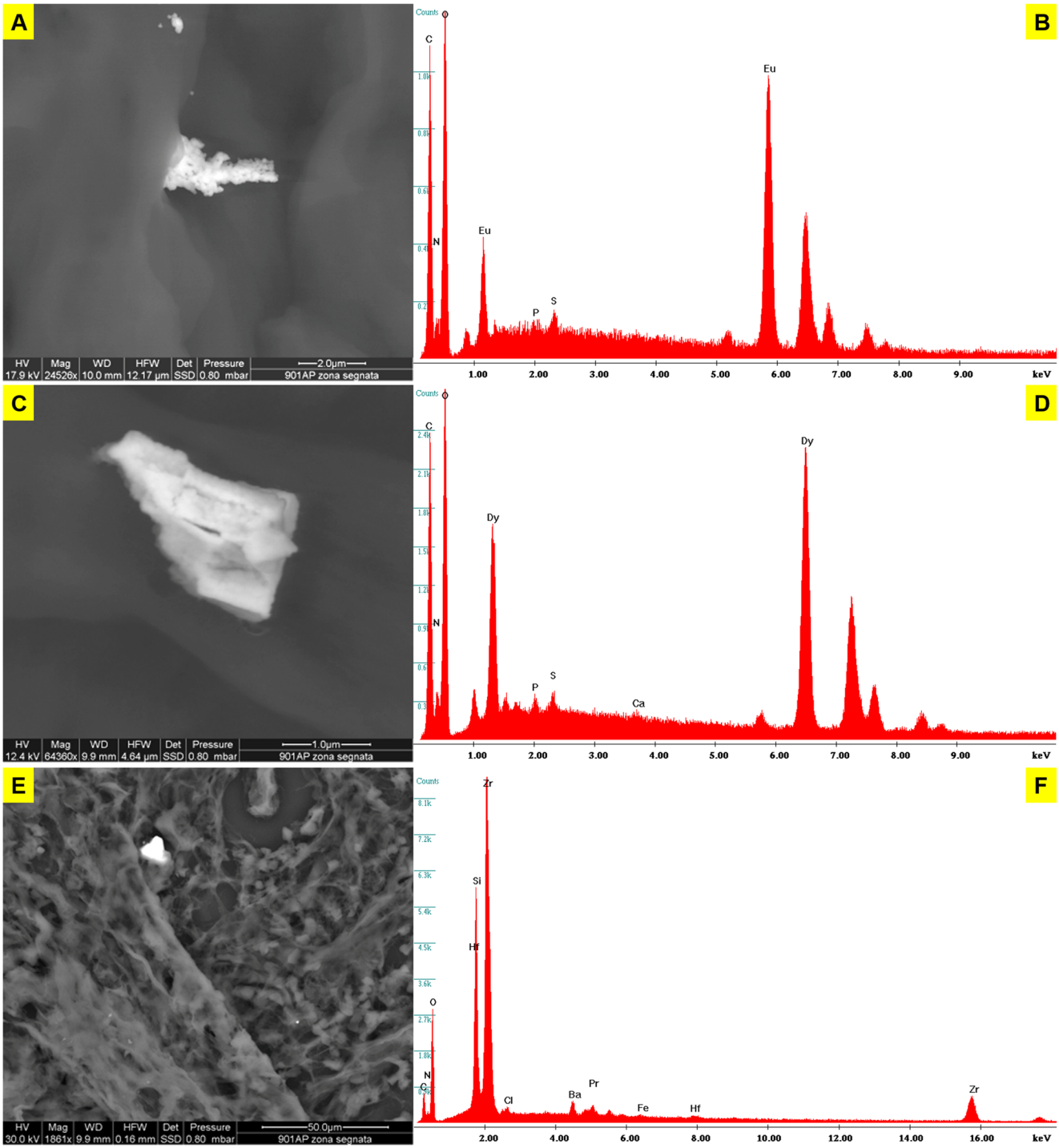

Fig. 2 ESEM image (**a**) with related EDS spectrum (**b**) of sub-micronic intratumoral debris, composed of europium (Eu) and organic elements, such as carbon (C), oxygen (O), azote (N), phosphorus (P) and sulphur (S); the high Eu peaks are well noticeable. ESEM image (**c**) with related EDS spectrum (**d**) of further intratumoral particulate matter, composed of dysprosium (Dy) and organic elements, such as carbon (C), oxygen (O), azote (N), phosphorus (P), sulphur (S) and calcium (Ca); the high Dy peaks are well noticeable. Finally, ESEM image (**e**) with related EDS spectrum (**f**) of another sub-micronic debris, composed of praseodymium (Pr), organic elements, such as carbon (C), oxygen (O), azote (N), chloro (Cl), ferrum (Fe), and inorganic salts, like silicon (Si), zirconium (Zr), hafnium (Hf) and barium (Ba), which testify a ceramic dust exposure; a low Pr peak is observable [X axis = keV; Y axis = counts]

in situ carcinoma. The immunohistochemical characterization has allowed to further subdivide the whole series into three diagnostic categories, according to the hormone receptor and Her2 status, as follows: ER+ / PgR+ / Her2+ (Her2 positive category, 29 cases), ER+ / PgR+ / Her2- (Her2 negative category, 57 cases), ER- / PgR- / Her2- (triple negative category,

27 cases). In only one high grade invasive ductal carcinoma (pT2N3a) of the triple negative category (Fig. 1), coming from a 78-year old female patient, who worked in the ceramic industry during her life, the microanalysis has significantly revealed the REM uptake by cancer cells (Fig. 2). More in detail, Eu, Dy and Pr have been detected together with silicon (Si), zirconium (Zr), hafnium (Hf) and barium (Ba), inorganic elements which testify a ceramic dust exposure (Fig. 2f), inside the neoplastic cells of the above mentioned triple negative cancer. After three years from the diagnosis, the tumor spread to lungs and pleura, causing the patient death. In all the remaining cases environmental particulate, mostly heavy metals, has been ascertained and its statistical impact on breast carcinogenesis is still in progress.

Discussion

For the first time in literature, we have demonstrated the *in vivo* uptake by breast cancer cells of three REM, more in detail Eu, Dy and Pr. The first two are medium REM, while the third is a light REM; no heavy REM have been found in our series. They have been detected inside the neoplastic cells coming from the ER- / PgR- / Her2- breast cancer of a female patient, who worked during her life in the ceramic industry, a well-known source of REM [9, 10]. From the anamnestic records, she was the one and only patient of our series to be dedicated to this activity. Their detection in 1 of 113 examined cases seems to exclude a crucial role in breast carcinogenesis, as also supported by other authors [11–13], and disclose new possibilities for therapeutic purposes. Eu and Dy have been detected with high microanalytic peaks, while Pr in trace level (Fig. 2): this finding suggests that medium REM have a special preference to be picked up by triple-negative breast cancer (TNBC) cells. Eu is the most reactive element among REM; it rapidly oxidizes when exposed to air and reacts in a manner similar to calcium in the presence of water [2]. Dy is relatively stable in air at room temperature and it quickly dissolves in dilute solutions of mineral acids, liberating hydrogen [2]. Eu and Dy powders present a fire and explosion hazard; moreover, they are characterized by a high capture cross section for neutrons and, for this reason, they have been used in the nuclear reactor control rods [2]. Just as Eu, Pr undergoes oxidation, when exposed to air, and becomes covered with a green veneer that protects it from further corrosion [2]. Nowadays, Eu, Dy and Pr have no known biological role and their mutagenicity has not been ever investigated. In the past, europium chloride, nitrate and oxide were tested for toxicity: europium chloride showed an acute intraperitoneal LD_{50} (median lethal dose) toxicity of 550 mg/kg and the acute oral LD_{50} toxicity was 5000 mg/kg [14]. Europium nitrate showed a slightly higher intraperitoneal LD_{50} toxicity of 320 mg/kg, while the oral toxicity was above 5000 mg/kg [15]. Soluble dysprosium salts, such as dysprosium chloride and dysprosium nitrate, were mildly toxic when ingested. The insoluble salts, however, resulted non-toxic. Based on the toxicity of dysprosium chloride to mice, it was estimated that the ingestion of 500 g or more could be fatal to a human being [15]. More recently, Giri and colleagues have patented 'nanoceria', a rare-earth nanoparticle as novel anti-angiogenic therapeutic agent in ovarian cancer [16]. TNBC is another well-known aggressive female tumor [17], accounts for approximately 15–25% of all breast cancer cases, it usually affects younger women, about the 75% are basal-type, and the standard treatments include surgery, adjuvant or neoadjuvant chemotherapy (taxanes, platinum-based agents) and radiotherapy [18]. However, TNBC shows a risk of relapse within the 5 years much higher than hormone-positive breast cancer [19]. Many current efforts have been focused to develop new therapeutic molecules and EGFR (epidermal growth factor receptor), PARP (poly ADP-ribose polymerase) or VEGF (vascular endothelial growth factor) inhibitors are constantly tested in experimental settings [19]. On average, TNBCs show significantly higher fluorine-18 fluorodeoxyglucose (FDG) uptake, measured by the SUV (standardized uptake values) max values, compared with the Her2 negative category, using FDG - positron emission tomography (FDG-PET) [20]. It is speculated that enhanced glycolysis in these tumors is related to their endocytic capacity and aggressive biology.

Conclusion

Based on the scientific evidence that TNBC cells show a pronounced endocytosis, together with our demonstration of the *in vivo* REM uptake by the same neoplastic cells, a rare-earth nanoparticle for the TNBC treatment might be thought, in order to offer a surviving chance for the patients affected by the more aggressive forms of this cancer.

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Compliance with Ethical Standards

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Conflict of Interest The authors declare no conflict of interest.

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