Correlation of Immunohistopathological Expression of Somatostatin Receptor-2 in Breast Cancer and Tumor Detection with ^{68}Ga-DOTATOC and ^{18}F-FDG PET Imaging in an Animal Model

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Abstract. *Background: Fludeoxyglucose positron emission topography (^{18}F-FDG PET) is insufficiently sensitive at detecting small or low-grade breast tumors. The characterization of somatostatin receptors (SSTR) in tumors and the development of ^{68}Ga-DOTATOC PET for imaging could be of interest. The aim of this study was to validate an animal model expressing SSTR2 and to correlate the immunohistochemical (IHC) analysis with ^{18}F-FDG and ^{68}Ga-DOTATOC uptake in vivo. Materials and Methods: Ten nude mice were xenografted with the ZR-75-1 breast tumor cell line. Imaging was performed with ^{68}Ga-DOTATOC and ^{18}F-FDG and correlated to IHC analysis of SSTR2. Results: IHC analyses showed that the tumors expressed SSTR2. On PET imaging, the tumors were barely visible with ^{18}F-FDG, whereas with ^{68}Ga-DOTATOC, specific two-fold higher uptake was observed ($p<0.005$). Conclusion: Our results suggest that ^{68}Ga-DOTATOC PET could be used for detection of breast tumors not detected with ^{18}F-FDG. SSTR2 status should be assessed to allow for individual treatment.*

Many studies have reported that ^{18}F-FDG positron emission topography (PET) has high sensitivity and specificity for the detection of various macroscopic malignant lesions, but breast cancer detection requires the ability to demonstrate non-palpable, small, invasive and *in situ* malignancies. These requirements are beyond the capacity of current whole-body ^{18}F-FDG PET examination, and thus ^{18}F-FDG PET is not well-adapted to primary breast cancer detection (1). In contrast, only one study, to our knowledge, has reported on breast cancer detection with ^{68}Ga-DOTATOC PET, incidentally in the context of whole-body PET performed for neuroendocrine tumors (2). ^{68}Ga-DOTATOC is a somatostatin analog designed for PET imaging which displays very high somatostatin receptor of type-2 (SSTR2) receptor binding. To date, no study has correlated the presence of SSTR2 in tumors to ^{68}Ga-DOTATOC PET imaging in breast malignancies. In neuroendocrine tumors, one study highlighted the correlation of SSTR2 expression with ^{68}Ga-DOTATOC PET (3).

Moreover, some recent studies have suggested the possibility of adding somatostatin analogs to the standard treatment for breast cancer (4-6). Thus, the presence of SSTR in the tumor and the correlation with *in vivo* uptake of ^{68}Ga-DOTATOC on PET could be useful in the staging and follow-up of patients with SSTR-positive tumors.

The aim of this preliminary study was to validate an animal model of breast tumor expressing SSTR2 and to correlate the immunohistochemical analysis to ^{18}F-FDG and ^{68}Ga-DOTATOC PET imaging.

Materials and Methods

Cell line and mouse inoculation. All *in vivo* experiments were performed in compliance with the French guidelines for experimental animal studies.

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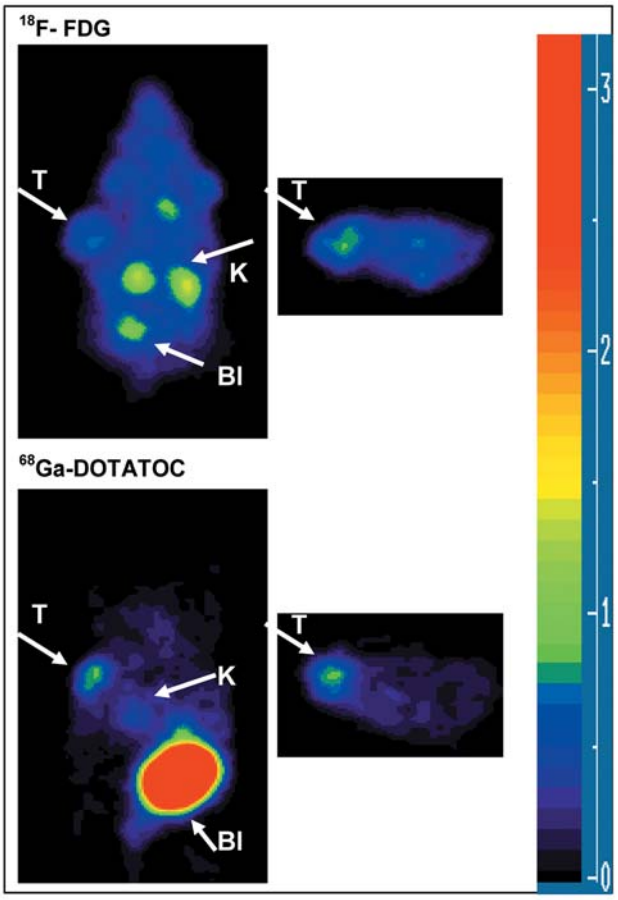

Figure 1. *Distribution of radioactive tracers in positron emission topography (PET) imaging: ^{18}F-FDG and ^{68}Ga-DOTATOC in a ZR-75-1 tumor model. Comparative PET imaging of the same female nude mouse grafted with ZR75-1 cells in the right flank, injected with ^{18}F-FDG or with ^{68}Ga-DOTATOC. Tumor volume was 400 mm^3. The acquisition time was 10 min, 1 h post-injection for ^{18}F-FDG, 45 min for ^{68}Ga-DOTATOC. Maximum intensity projection (MIP) or axial frame. Bl: Bladder. K: Kidney. T: Tumor.*

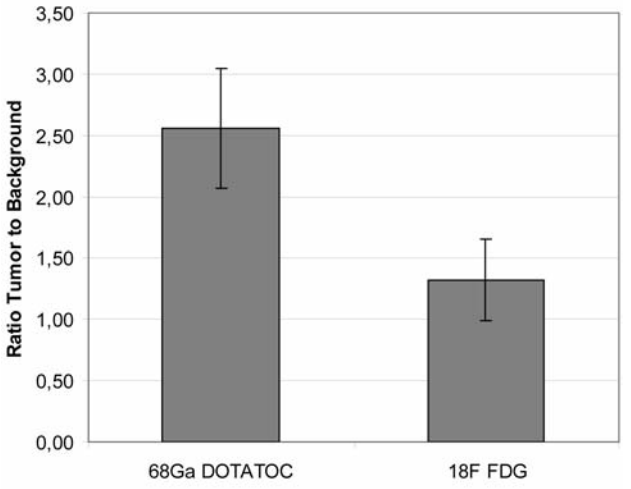

Figure 2. *Tumor-to-background ratio for ^{18}F-FDG and ^{68}Ga-DOTATOC in a ZR-75-1 tumor model. Quantitative micro positron emission topography (PET) ROI analysis of tumor uptake. Data are expressed in uptake ratios (tumor to non tumor) calculated by Syntegra in standardized uptake value (SUV) max±SD.*

We used the ZR-75-1 cell line, known to express SSTR2 (7), which was provided by ATCC (LGC Standards Sarl, Molsheim, France). This cell line was obtained from human invasive ductal breast carcinoma, and it also expresses estrogen receptors.

Cells were incubated and mixed with Matrigel solution before subcutaneous inoculation (5×10^6 cells under anesthesia with 1.5% isoflurane) into the right part of the abdomen of 10 nude mice (Charles Rivers, France). The mice were prepared with a subcutaneous implant of estrogen under the neck one week before cell inoculation (IRA, Sarasota, FL, USA), to enhance cell division and tumor growth.

Tumor volume (V) was assessed every two days by caliper measurement as follows: $V=ab^2\pi/6$, where a is the longer, and b is the shorter of two perpendicular diameters. PET imaging was performed when the tumors had grown to 500 mm^3.

^{68}Ga-DOTATOC radiolabelling. A fully-automatic, PC-controlled, radiopharmaceutical synthesis device (SynChrom R&D, Raytest, Germany) was used for all steps of the radiolabelling. ^{68}Ga ($t_{1/2}$ 68 min) was eluted using a ^{68}Ge/^{68}Ga-generator-system (IGG100, Eckert Ziegler, Berlin, Germany) by a fractionated method with 5 ml of 0.1 M hydrochloric acid. DOTATOC (17 nmol, 1 μg/ml, Iason, Austria), in 800 μl of 0.8 M sodium acetate, was added to the most concentrated fraction of 150 MBq of $^{68}GaCl_3$ in 2.3 ml of 0.1 M HCl. The reaction mixture (pH 4) was incubated at 95˚C for 8 min. Excess free ^{68}Ga was removed onto a Sep-Pak cartridge (Waters Milford, USA). Radiochemical purity was confirmed by reverse-phase high-performance liquid chromatography (HPLC).

PET imaging. PET acquisitions were performed using a Mosaic animal PET machine (Philips Medical Systems, Cleveland, OH, USA).

For each mouse, comparative imaging was performed with ^{18}F-FDG and ^{68}Ga-DOTATOC. Briefly, for ^{18}F-FDG PET, after a fasting period of 12 h, the mice were injected *i.v.* in the retro-orbital sinus with 5 to 10 MBq ^{18}F-FDG, and they underwent imaging one hour later. Two days later, the same animals were injected with 2.95±0.72 MBq ^{68}Ga-DOTATOC (870±70 pmol) and were imaged 45 min later. Static acquisitions were performed with an exposure time of 10 min. The data were standardized in standardized uptake value (SUV units, SUVmax) and were analyzed using the Syntegra-Philips software. The maximum SUVs in tumors were determined and reported.

The mice were sacrificed after imaging by cervical dislocation, and the tumors were removed, weighed and formalin-fixed.

Immunohistochemistry. The tumors were embedded in paraffin according to standard procedures. Thin paraffin sections of 4 μm were immunostained with antibodies against SSTR2 (RBK046-05,

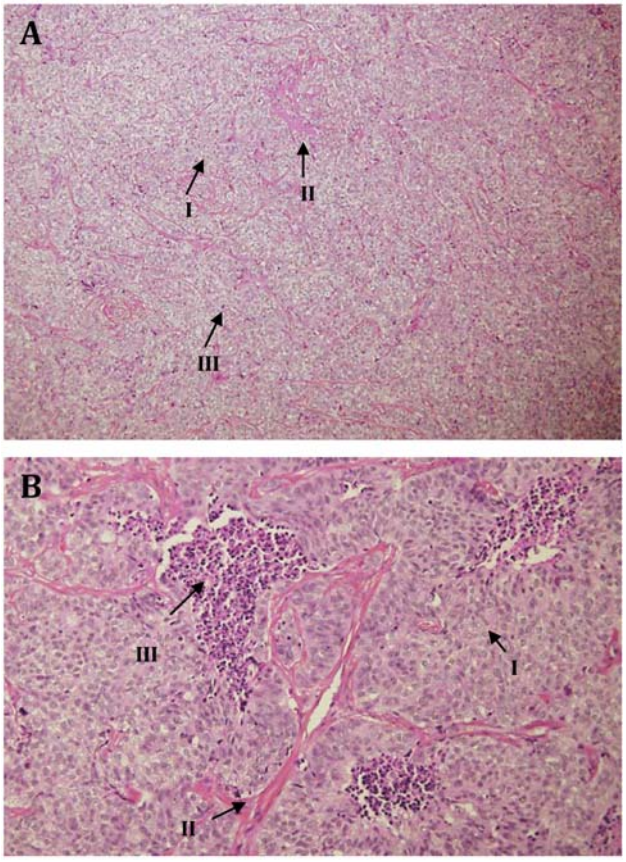

Figure 3. *Hematoxylin and Eosin (HES) coloration of a tumor obtained from the ZR-75-1 cell line. The slides were imaged using an upright microscope, in transmitted light (Nikon Eclipse). A: Structural organization of tumor tissue (magnification 10×10 microns wide): tumor cells (I), tissue (II), inflammatory cells (III). B: Magnification of an inflammatory focus (II) surrounded by tumor cells (I) (magnification ×20, scale bar: 100 microns).*

Zytomed Systems®) after deparaffinization and protease-based antigen retrieval, on an automated immunostainer using the ABC method. We used paraffin sections of human pancreas for positive control staining.

We also studied tumor morphology with Hematoxylin and Eosin (HES) staining to assess histological tumor characteristics.

Statistical analysis. Data were analyzed using the Chi-square test or the Fisher's exact test and the Student's *t*-test. Differences were considered significant when $p<0.05$.

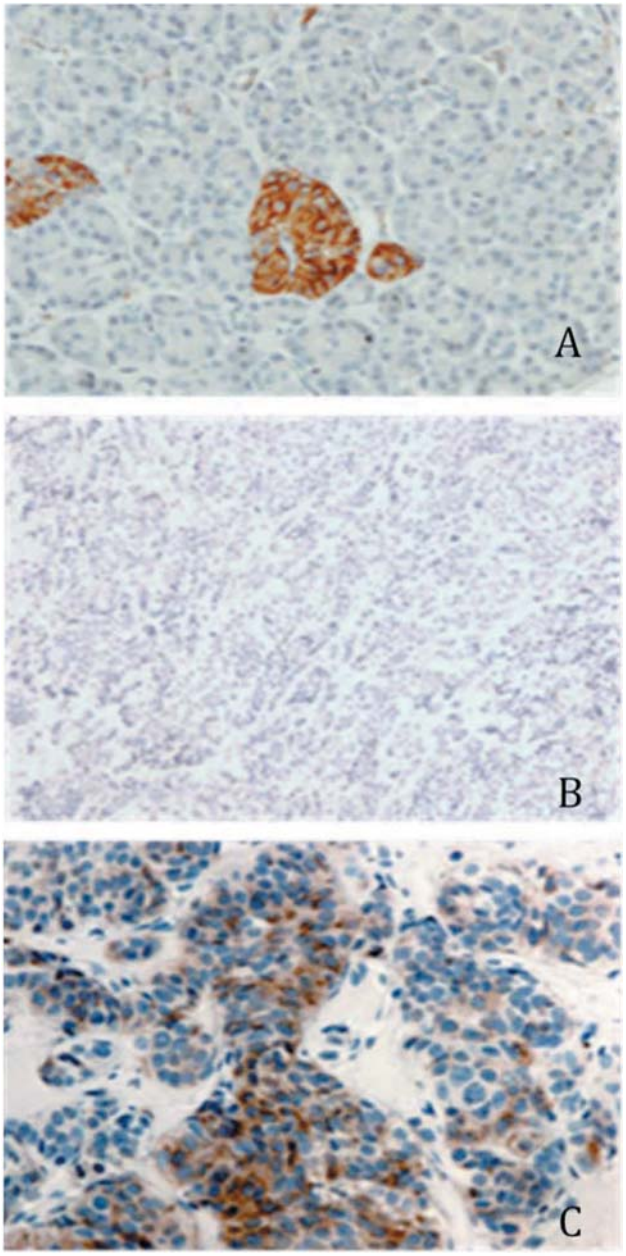

Figure 4. *Immunohistochemistry using somatostatin receptor of type-2 (SSTR2) antibody. The images were observed under an optical microscope in transmitted light (Nikon Eclipse) (magnification ×20, scale bar: 100 microns). A: Human pancreas. B: Negative sampling without antibody. C: Tumor obtained from the ZR-75 -1 cell line.*

Results

Radiolabelling. The overall preparation time was 30 min. After purification of the labeled compound on a reverse-phase C18 cartridge, the radiochemical purity of ^{68}Ga-DOTATOC, checked by HPLC, was 100%. The decay-corrected labeling yield of ^{68}Ga-DOTATOC obtained was 88.3%, with a specific activity of 5.41 MBq/nmol at the end of the labeling process.

PET imaging. We studied 10 nude mice bearing ZR-75-1 tumors. On ^{18}F-FDG PET, we observed the expected bio-

distribution, with uptake in brown neck fat, in the brain and in the heart and elimination of the tracer through the urinary system.

With ^{68}Ga-DOTATOC, the kidneys and bladder were observed on PET imaging, reflecting the dominant clearance of ^{68}Ga-DOTATOC from the body. Projections and axial frames distinctly showed the ZR75-1 xenograft (Figure 1).

The tumors were barely visible with ^{18}F-FDG, while they appeared with a moderate but definite uptake on ^{68}Ga-DOTATOC PET. Uptake of ^{68}Ga-DOTATOC in ZR75-1 tumors was two-fold greater than that of ^{18}F-FDG (2.56±0.5 *vs*. 1.3±0.3, respectively; $p<0.005$; Figure 2).

Immunohistochemistry. HES histologic analysis confirmed that inoculation of ZR-75-1 cells resulted in tumors with the expected characteristics (Figure 3). The IHC analysis showed that the tumors expressed SSTR2 (Figure 4). The control sample of human pancreas showed staining with SSTR2 antibody. Indeed, the human pancreatic endocrine islets were clearly delineated with this antibody (Figure 4A). The staining of ZR-75-1 tumors by the SSTR2 antibody was characteristic of this type of receptor. The labeling was more intense on the periphery of the cells and was less distinct, or even absent, inside the cells. The darker grains in the cytoplasm corresponded to the routing of receptors on the cell membrane (Figure 4C).

Discussion

In this study, we showed that positive ^{68}Ga-DOTATOC PET imaging was correlated with positivity for SSTR2 immunostaining. While this animal model of breast cancer could not be visualized on ^{18}F-FDG imaging, we demonstrated that it can be visualized when a somatostatin analog PET is performed.

We can therefore conclude that ^{68}Ga-DOTATOC imaging was more efficient than ^{18}F-FDG in detecting breast tumors expressing SSTR2 in this animal model. To our knowledge, this is the first study that combined immunohistochemical detection of SSTR2 and ^{68}Ga-DOTATOC PET in an animal model of breast cancer.

In a previous study, Elegeti *et al*. retrospectively analyzed whole-body ^{68}Ga-DOTATOC PET performed for the staging of neuroendocrine tumors (2). In this series of 33 patients, they observed two synchronous breast carcinomas.

Samson *et al*. performed a meta-analysis of 13 articles evaluating whole-body FDG PET in breast cancer detection (8). On the basis of this analysis, FDG PET was 88% sensitive and 80% specific for breast cancer with false-negative results in 12% of cancer cases. Both tumor size less than 10 mm and low tumor grade were significant predictors of a false-negative FDG PET result. The authors concluded that whole-body FDG PET should not be used to characterize breast lesions, and they also noted that most studies evaluating FDG PET in breast cancer were unevenly weighted towards large, palpable primary lesions, typically omitting non-palpable, imaging-detected carcinomas, which are a critical segment of the biopsy population.

The presence of SSTR2 has been evaluated in breast cancer. Orlando *et al*. found, in a series of 169 breast cancer cases, that all of the tumors expressed *SSTR2* mRNA (9). They found that the absolute concentrations of *SSTR2* mRNA were significantly higher in estrogen receptor-positive cases, in lymph node-negative cancers, in patients with T1 disease and in low-proliferating breast carcinomas. They also found that up-regulation of the *SSTR2* gene expression was associated with a better prognosis.

The use of somatostatin analogs in the treatment of breast cancer remains controversial, and the results available in the literature have been discordant (10). For metastatic breast cancer, a randomized study comparing the administration of tamoxifen combined with octreotide to tamoxifen associated with placebo in patients with recurrent or metastatic cancer did not find significant differences between the two groups regarding progression-free survival (4). A literature review reporting on 210 patients included in 40 studies (published or unpublished), treated for metastatic breast cancer with somatostatin analogues, showed an improvement in progression-free survival, especially in patients with first-line metastatic tumors and in patients with fewer than two metastases (5). In an adjuvant setting, one recent study, based on 667 patients using associated tamoxifen-octreotide *vs*. tamoxifen alone, did not show any difference between the two groups regarding overall or disease-free survival (6).

Featuring a possible role for somatostatin analogs in combined endocrine therapies for breast cancer, our results suggest that the SSTR2 status of tumors should be investigated.

Conclusion

These results suggest that the ^{68}Ga-DOTATOC PET could be used in this setting for the imaging of low-grade breast carcinomas not detected with ^{18}F-FDG.

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