

Article

99mTc-Sestamibi bioaccumulation can induce the apoptosis in breast cancer cells: molecular and clinical perspectives

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Abstract: The aim of this study was to investigate the possible role of 99mTc-sestamibi in the regulation of cancer cell proliferation and apoptosis. To this end, *in vivo* values of 99mTc-sestamibi uptake have been associated to the *in-situ* expression of both Ki67 and caspase-3. For *in vitro* investigations BT-474 cells were incubated with three different concentration of 99mTc-sestamibi: 10µg/ml – 1µg/ml – 0,1µg/ml. Expression of caspase-3 and Ki67, as well as the ultrastructure of cancer cells, were evaluated at T0 and after 24, 48, 72 and 120 hours after 99mTc-sestamibi incubation. *Ex vivo* data strengthened the known association between the sestamibi uptake and the Ki67 expression. Linear regression analysis showed a significant association between the sestamibi uptake and the number of apoptotic cells evaluated as caspase-3 positive breast cancer cells. As concern the *in vitro* data, a significant decrease of the proliferation index was observed in breast cancer cells incubated with high concentration of 99mTc-sestamibi (10µg/ml). Amazingly, a significant increase in caspase-3 positive cells in cultures incubated with 10µg/ml 99mTc-sestamibi was observed. This study suggested the possible role of sestamibi in the regulation of pathophysiological process involved in breast cancer.

Keywords: 99mTc-Sestamibi; Breast Specific Gamma Imaging; Breast Cancer; Apoptosis; Theragnostic

1. Introduction

A recent report of The American College of Radiology releases the clinical indications for the use of dedicated breast gamma imaging, including Breast Specific Gamma Imaging (BSGI), in breast cancer patients not suitable for the Resonance Imaging (MRI) analysis [1]. Indeed, BSGI with the cationic lipophilic agent Technetium 99-m labeled sestamibi (99mTc-sestamibi) is considered a reliable and useful medical device for the early preoperative detection of primary breast cancer [2]; BSGI with 99mTc-sestamibi can be also considered complementary to mammography in women showing dense breasts, palpable abnormalities and mammographically indeterminate breast lesions >1 cm [3]. For all these reasons, the correct use of BSGI with 99mTc-sestamibi in the breast Units frequently allows to reduce the number of unnecessary biopsies thus improving the

management of breast cancer patients. Moreover, it is established a role of BSGI with ^{99m}Tc -sestamibi for accurate stereotactic breast biopsy procedure. In fact, Collarino and colleagues [4] described a new clinical method for stereotactic breast biopsy guided by ^{99m}Tc -sestamibi uptake that allows the achievement of larger and suitable histopathologic samples, if compared with automated core needle biopsy methods [5,6].

Recently, Urbano et al. demonstrated that BSGI with ^{99m}Tc -sestamibi can be used to detect breast cancer lesions characterized by the presence of microcalcifications and breast osteoblast-like cells (BOLCs) [7]. Indeed, authors showed a significant association among the ^{99m}Tc -sestamibi uptake, the presence of breast microcalcifications made of hydroxyapatite and the presence of cancer cells associated to the development of bone metastatic lesions (BOLCs) [8]. Thus, BSGI with ^{99m}Tc -sestamibi could also be used to early detection of breast cancer lesions with high propensity to form bone metastasis [9].

As concern the ^{99m}Tc -sestamibi uptake, three different biodistribution models based on its chemical-physical characteristics have been proposed [10-12]: a) binding of ^{99m}Tc -sestamibi with 8–10-kDa cytoplasmic proteins, b) easy lipid partitioning and membrane translocation mainly associated to passive transmembrane distribution in accordance with the imposed transmembrane potential. The passive membrane translocation is currently the most supported mechanism [13-14]. Based on this mechanism the uptake of sestamibi is generally associated to the presence of several mitochondria with an high membrane potential [15].

Despite the important chemical-physical and clinical evidences about the use of BSGI with ^{99m}Tc -sestamibi in the management of breast cancer patients reported above, few studies have been performed on the cellular/molecular modifications of breast cancer tissues induced by the sestamibi uptake. In fact, only indirect data about the association between ^{99m}Tc -sestamibi uptake and cellular/molecular characteristics of breast cancer cells, such as proliferation index, have been investigated. In this context, Erba et al. reported very preliminary data about the role of sestamibi uptake as indicators of chemotherapy induced apoptosis [16]. However, no definitive clinical or in vitro experimental data is currently available.

Starting from these considerations, this study aims to investigate both ex vivo and in vitro the possible role of ^{99m}Tc -sestamibi in the regulation of biological process involved in cancer progression such as proliferation and apoptosis. To this end, the in vivo values of ^{99m}Tc -sestamibi uptake have been associated to the in-situ expression of both Ki67 (proliferation index) and caspase 3 (apoptosis). In addition, in vitro investigations using a breast cancer cells line (BT474) have been performed to study the possible cellular and molecular modifications of cancer cells following the ^{99m}Tc -sestamibi uptake.

2. Materials and Methods

“Policlinico Tor Vergata” Ethical Committee approved this protocol with the reference number # 129.18, 26 July 2018. Also, all methodologies and experimental procedures here described were achieved in agreement with the last Helsinki Declaration.

Exclusion criteria were a second cancer and neoadjuvant hormonal or radiation therapy prior to surgery.

According to these criteria, we retrospectively enrolled 40 consecutive breast cancer patients (58.36 ± 1.99 years; range 42–65 years) underwent both BSGI with ^{99m}Tc -Sestamibi and breast bioptic procedure.

For each of them, histological diagnosis and immunohistochemical investigations were performed.

99mTc-Sestamibi -High Resolution SPECT

BSGI with 99mTc-Sestamibi investigations were performed as described in a previous study [17]. Briefly, BSGI scan was performed in 10–15 min following an intravenous administration of 740 MBq 99mTC-Sestamibi through an antecubital vein contralateral to the suspicious breast side to avoid potential false-positive uptake in the axillary lymph nodes. The patients remained seated during the procedure. Craniocaudal and mediolateral oblique (MLO) images were obtained in both breasts using a high-resolution BSGI.

All 40 patients had biopsy. BSGI was performed before biopsy in 25 patients and after biopsy in 15 patients. When BSGI was performed after biopsy, the minimum interval between biopsy and imaging was 7 days in an effort to avoid the effects of post-biopsy inflammation as much as possible.

For qualitative analysis of BSGI, two investigators classified positive and negative findings. Lesions with no demonstrable uptake and those with diffuse heterogeneous or minimal patchy uptake were considered negative whereas lesions with scattered patchy uptake, partially focal uptake, or any other focal uptake were regarded as positive. Irregular-shaped regions of interest (ROIs) were used to encase the lesions. The evaluation of the lesion to nonlesion ratio (L/N) were estimated according to the study of Tan et al. [18]. For patients underwent BSGI with 99mTC-sestamibi before biopsy (n=25), the BSGI-guided biopsy procedure was performed as previously described [19].

Histology

Breast bioptic samples were formalin fixed and embedded in Paraffin. Serial sections were used for both hematoxylin-eosin (H&E) and immunohistochemistry staining [20].

Immunohistochemistry

Immunohistochemistry was used to study the expression of an apoptotic in situ biomarker, the Caspase 3, and the proliferation index by Ki67. Three- μ m-thick paraffin sections were treated with EDTA citrate buffers pH 7.8 for 30 min at 95°C to antigen retrieval reaction. Afterwards, sections were incubated with the primary antibodies diluted 1:100 for 60 minutes at room temperature; anti-Ki67 rabbit monoclonal antibody (clone 30-9, Ventana, Tucson, USA) and anti-caspase 3 mouse monoclonal antibody (31A1067, Novus Biologicals, USA). Washing was performed with PBS/Tween 20 pH 7.6 (UCS Diagnostic).

Digital scan was used to evaluate the immunohistochemical reactions (Iscan Coreo, Ventana, Tucson, AZ, USA). Specifically, digital images from caspase 3 reactions were evaluated in a semi-quantitative approach by counting the number of positive breast cancer cells (out of a total of 500 in randomly selected regions). Ki67 was calculated in terms of percentage of positive cancer cells. Reactions have been set-up by using specific positive and negative control tissues.

Cell Culture

BT-474 cells obtained from the American Type Culture Collection (ATCC, Manassas, Virginia, USA) and maintained by the Cell and Tissue Culture Core, Lombardi Cancer Center (Reservoir Rd. NW Washington D.C. 20057, USA). Cells were routinely cultured in DMEM high glucose (Sigma-Aldrich, St. Louis, Missouri, USA) supplemented with 10% fetal bovine serum (FBS).

In detail, cells from the first or second passage were seeded into a 24-well plate at a density of 30×10^3 cells/well. Successively, BT-474 cells were incubated with: (a) 99mTc-sestamibi 10 µg/ml (b) 99mTc-sestamibi 1 µg/ml and (c) 99mTc-sestamibi 0.1 µg/ml. The expression of both Ki67 and caspase 3 were evaluated at T0 and after 24, 48, 72 and 120 hours after sestamibi incubation. Cells treated with the vehicle were used as control (CTRL).

Cell proliferation was investigated by both counting the number of cells for each time point and bromodeoxyuridine Incorporation Assay performed at time 0 and after 72 h. Morphology was studied by both toluidine blue staining.

Immunocytochemistry

Immunocytochemistry was performed to investigate the expression of caspase 3 and Ki67 on BT-474 cells treated with 99mTc-Sestamibi. Caspase 3 was evaluated by immunoperoxidase analysis whilst Ki67 expression was evaluated by immunofluorescence staining in order to reduce the background.

BT-474 Cells were plated on poly-L-lysine coated slides (Sigma-Aldrich cat #P4707) in 24-well cell culture plates and fixed in 4% paraformaldehyde. After pre-treatment with EDTA citrate at 95 °C for 20 min and 0.1% Triton X-100 for 15 min, cells were incubated 1 h with the anti-Ki67 rabbit monoclonal antibody (clone 30-9, Ventana, Tucson, USA) and anti-caspase 3 mouse monoclonal antibody (31A1067, Novus Biologicals, USA). Washings were performed with PBS/Tween20 pH 7.6. As concern the study of caspase 3 expression, reactions were revealed by the HRP-DAB Detection Kit (UCS Diagnostic, Rome, Italy). Conversely, the Ki67 signal was revealed by using an TexasRed conjugate anti-rabbit antibody.

Reactions were evaluated by counting the number of Ki67 or caspase 3 positive cells on 500 in total in randomly selected regions.

TEM and EDX Analysis of Cell Cultures

Cells were fixed in 4% paraformaldehyde, post-fixed in 2% osmium tetroxide and embedded in Epon resin for morphological studies. After washing with 0.1 M phosphate buffer, the sample was dehydrated by a series of incubations in 30%, 50%, and 70% ethanol. Dehydration was continued by incubation steps in 95% ethanol, absolute ethanol, and hydroxypropyl methacrylate, then samples were embedded in Epon (Agar Scientific, Stansted Essex, UK).

Eighty µm ultra-thin sections were mounted on copper grids and observed with Hitachi 7100FA transmission electron microscope (Hitachi, Schaumburg, IL, USA) to study the mitochondria ultrastructure.

Unstained ultra-thin sections of approximately 100-nm-thick were mounted on copper grids for microanalysis. EDX spectra were acquired with a Hitachi 7100FA transmission electron microscope (Hitachi, Schaumburg, IL, USA) and an EDX detector (Thermo Scientific, Waltham, MA, USA).

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 5 Software (San Diego, CA, USA). Immunohistochemical data were analyzed by the Kruskal–Wallis test ($p < 0.05$) and by Mann–Whitney test ($p < 0.0005$). In vitro data about the number of Ki67 positive cells and caspase-3 positive cells were analyzed by using one-way ANOVA ($p < 0.05$).

3. Results

99mTc-Sestamibi-High Resolution SPECT Analysis

BSGI with 99mTc-Sestamibi shown the uptake of the radiopharmaceutical in all 35 breast cancer patients (L/N max 5,09; min 1,43) (Fig.1A). No significant differences concerning L/N Ratio were observed among breast cancer histotypes.

Histology

Breast biopsies were classified according to Nottingham Histological system [21]. In particular, 8/40 G1 infiltrating carcinomas, 14/40 G2 infiltrating carcinomas and 9/40 G3 infiltrating carcinomas were found.

99mTc-Sestamibi Uptake vs. apoptosis

To investigate the possible association between the sestamibi uptake and the apoptotic phenomenon linear regression analyses have been performed by comparing L/N Ratio values and the number of caspase 3 positive cells. Linear regression analysis showed a positive association between the sestamibi uptake and the number of caspase 3 positive breast cancer cells ($p=0.0424$; $r^2 0.1190$) (Fig.1B,D,E), even though the absolute number of apoptotic cells were low especially if compared with the number of Ki67 positive cells (Fig.1C,D).

99mTc-Sestamibi Uptake vs. proliferation index

The percentage of Ki67 positive breast cancer cells, evaluated by immunohistochemistry, has been used as proliferation index values. According to our previously investigation, a positive and significant correlation between the sestamibi uptake and proliferation index was observed ($p<0.0001$; $r^2 0.5685$) (Fig.1C,F).

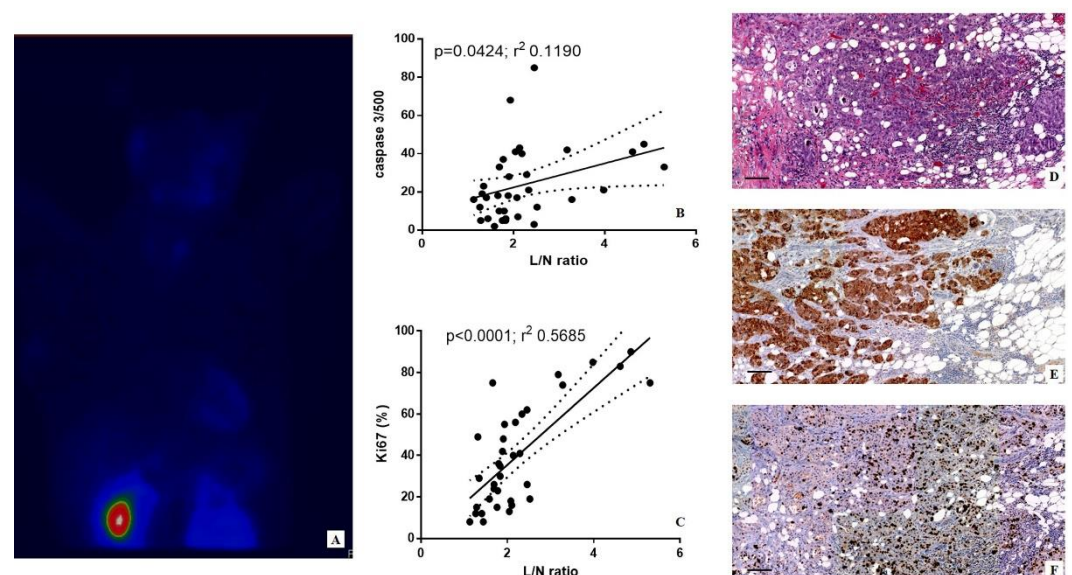


Figure 1. Breast Specific Gamma Imaging with 99mTc-sestamibi and both caspase 3 and ki67 expression. A) Maximum-intensity projection of Breast Specific Gamma Imaging with 99mTc-sestamibi in a 69-year-old breast cancer patient. B) The graph shows the positive and significant association between the number of caspase 3 positive breast cancer cells and the sestamibi uptake (L/M ratio). C) The graph displays significant association between the number of Ki67 positive breast cancer cells and the sestamibi uptake (L/M ratio). D) Hematoxylin and eosin section shows an G3 infiltrating breast carcinomas (scale bar represents 50µm). E) Image displays numerous

caspase 3 positive breast cancer cells (scale bar represents 50 μ m). F) High percentage of Ki67 positive breast cancer cells (scale bar represents 50 μ m).

Effect of sestamibi on breast cancer cells: in vitro study

At the end of each experimental point, BT-474 cells were fixed with 4% paraformaldehyde and used to evaluate the number of both Ki67 and caspase 3 positive cells. One-way ANOVA showed significant data distribution for both the number of caspase 3 ($p<0.0001$) and Ki67 ($p=0.0038$) positive cells. Noteworthy, high concentration of 99mTC-sestamibi (10 μ g/ml) induced a significant increase in the number of apoptotic cells (caspase 3 positive cells) if compared with all others experimental conditions, included the controls (Fig.2A-I). Specifically, a great increase in the number of caspase 3 positive cells was observed after 48h (Fig.2A,C). This datum suggests that only at high concentration the sestamibi could be able to induce the apoptotic process by caspase 3 signal. In other experimental condition, no significant effects were detected (Fig.2A).

As concern the cell proliferation (Ki67), a significant decrease in the number of Ki67 positive cells was observed in cell cultures incubated with both 10 μ g/ml and 1 μ g/ml of 99mTC-sestamibi if compared with cell treated with an concentration of 0.1 μ g/ml (Fig.2M-P). Indeed, data here reported showed that low concentration of 99mTC-sestamibi (0.1 μ g/ml) does not influence the proliferation index (Fig.2M). Cell cultures treated with both 1 μ g/ml and 0.1 μ g/ml 99mTC-sestamibi displayed a proliferation index similar to that observed in the controls (Fig.2M). This datum can explain the association observed in vivo between the sestamibi uptake and the percentage of Ki67 positive cells. It is important to note that high concentration of 99mTC-sestamibi (10 μ g/ml) induced a significant reduction of the proliferation index already after 24h of treatment (Fig.2M-L).

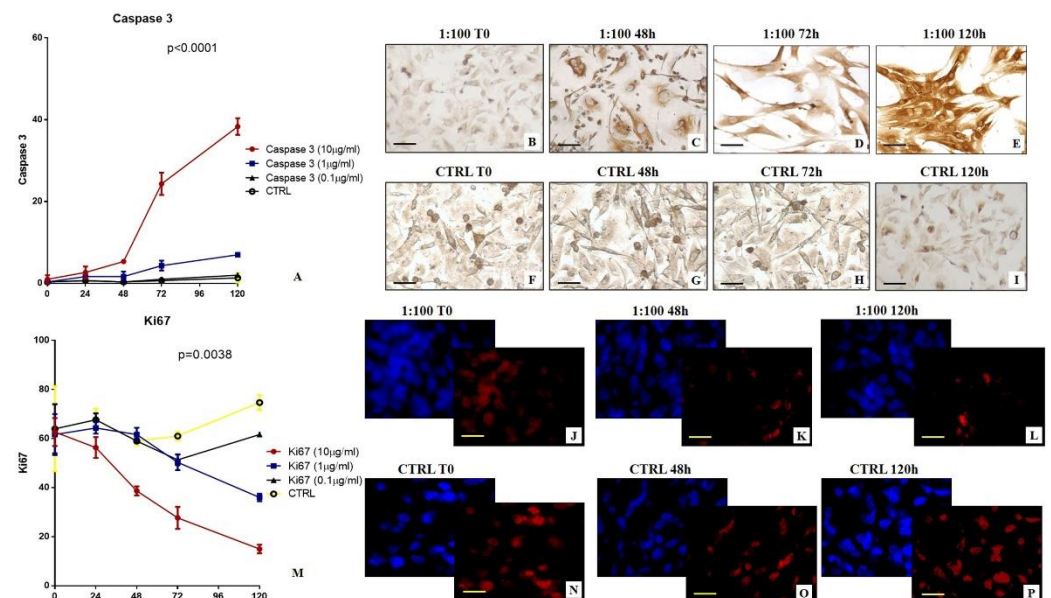


Figure 2. In vitro evaluation of the effect of 99TC-sestamibi on BT474 breast cancer cells. A) The graph shows the number of caspase 3 positive BT474 breast cancer cells after sestamibi treatment. B) No/rare caspase 3 positive cells at T0. C) Image shows caspase 3 positive cells after 48 hours of 99TC-sestamibi incubation (10 μ g/ml). D) Numerous caspase 3 positive cells after 72 hours of 99TC-sestamibi incubation (10 μ g/ml). E) High number of caspase 3 positive cells after 120 hours of 99TC-sestamibi incubation (10 μ g/ml). F,G,H,I) No/rare caspase 3 positive cells at each time point in absence of 99TC-sestamibi incubation. Scale bar represents 50 μ m for B,C,D,E,F,G,H,I images. M) The graph shows the number of caspase 3 positive BT474 breast cancer cells after sestamibi treatment. J) Immunofluorescence displays numerous Ki67 positive breast cancer cells (blue DAPI –Texas red ki67) at T0. K) Some Ki67 positive breast cancer cells (blue DAPI –Texas red Ki67)

after 48 hours of ^{99}Tc -sestamibi incubation ($10\mu\text{g/ml}$). L) Rare Ki67 positive breast cancer cells (blue DAPI –Texas red Ki67) after 120 hours of $10\mu\text{g/ml}$ ^{99}Tc -sestamibi incubation. N,O,P) Numerous Ki67 positive breast cancer cells (blue DAPI –Texas red Ki67) at each time point in absence of ^{99}Tc -sestamibi incubation ($10\mu\text{g/ml}$). Scale bar represents $20\mu\text{m}$ for J,K,L,N,O,P images.

By comparing the value of Ki67 and caspase 3 at every time point it became clear that only using the $10\mu\text{g/ml}$ of ^{99}mTc -sestamibi concentration a complete reversion between proliferation and apoptosis was obtained (Fig.3A-C). In particular, after 72h the number of caspase 3 positive cells were higher than those positive for Ki67 suggesting an imbalance capable to arrest the tumour proliferation (Fig.3A). Morphological evaluation confirmed the presence of apoptotic bodies in the cell cultures cultured with high concentration of ^{99}mTc -sestamibi ($10\mu\text{g/ml}$) (Fig.3D-G). Transmission electron microscopy investigations showed numerous apoptotic bodies in cell cultures incubated with $10\mu\text{g/ml}$ ^{99}mTc -sestamibi (Fig.3E-G). The damages were evident after 72h. EDX microanalysis frequently showed the presence of technetium in the cytoplasm of apoptotic cells (Fig.3F,G) or in the mitochondria of cell in the early phases of apoptotic process.

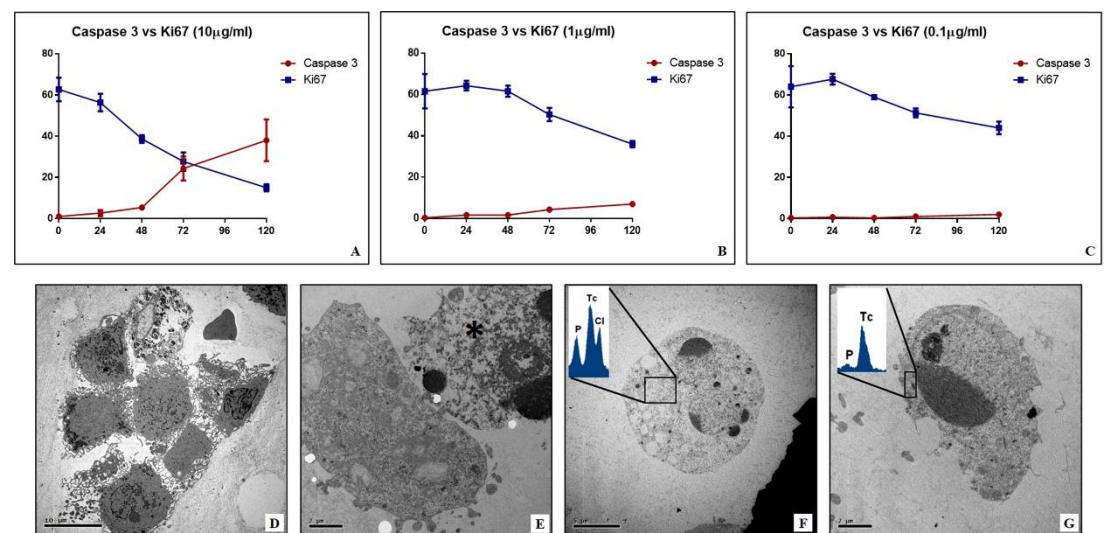


Figure 3. Comparison between apoptosis and proliferation of BT474 breast cancer cells treated with ^{99}Tc -sestamibi and ultrastructural analysis. A) Graph shows the number of both caspase 3 and Ki67 positive BT474 breast cancer cells after 24, 48, 72, 96 and 120 hours of ^{99}Tc -sestamibi incubation ($10\mu\text{g/ml}$). B) Graph displays the number of both caspase 3 and Ki67 positive BT474 breast cancer cells after 24, 48, 72, 96 and 120 hours of ^{99}Tc -sestamibi incubation ($10\mu\text{g/ml}$). C) Graph shows the number of both caspase 3 and Ki67 positive BT474 breast cancer cells after 24, 48, 72, 96 and 120 hours of ^{99}Tc -sestamibi incubation ($10\mu\text{g/ml}$). D) Electron micrograph displays ultrastructure of BT474 breast cancer cells (CTRL T0). E) Image shows an apoptotic breast cancer cells (asterisk) close to a non-apoptotic cell ($10\mu\text{g/ml}$ at 48 hours). F,G) EDX spectrum reveals the presence of Technetium into the cytoplasm of apoptotic cells ($10\mu\text{g/ml}$ at 120 hours).

4. Discussion

BSGI with ^{99}mTc sestamibi can represent an remarkable opportunity to detect both primary and metastatic lesions characterized by the presence of breast cancer cells with high amount of mitochondria. In fact, the currently data about chemical-physical characteristics of sestamibi, as well as its pharmacokinetics, seem to indicate its tropism for the accumulation into the cell organelles with a negative membrane potential such as mitochondria. A support of this, it is known that once in the cytoplasm, sestamibi may translocate into mitochondria according to its cationic nature [15,16]. The positive charge on sestamibi may drive this molecule into mitochondria during cell metabolic activities that increase the negative plasma membrane potential. Despite these evidences, rare studies have been performed about the possible effect of sestamibi uptake in breast cancer cells. The accumulation of sestamibi into the cell allows to hypothesize

the alteration of normal mitochondria functions and consequently of the cancer cell's homeostasis, mainly at the level of cell proliferation and cell death.

According to this idea, this study aims to investigate both *ex vivo* and *in vitro* the possible role of ^{99m}Tc-sestamibi in the regulation of biological process involved in cancer progression. To this end, the *in vivo* values of ^{99m}Tc-sestamibi uptake have been associated to both the *in-situ* expression of Ki67 (proliferation index) and caspase-3 (apoptosis). In addition, *in vitro* investigations using a breast cancer cells line (BT-474) have been performed to study the possible cellular and molecular modifications of cancer cells following the ^{99m}Tc-sestamibi uptake.

Here reported *ex vivo* data strengthened the known association between the sestamibi uptake and the Ki67 expression thus confirming the capability of BSGI with ^{99m}Tc sestamibi to identify breast cancer lesions characterized by high proliferation index [17]. In line with this, Urbano et al. also demonstrated the association between sestamibi uptake and proliferation index in parathyroid lesions [18]. Surprisingly, linear regression analysis also showed a significant association between the sestamibi uptake and the number of apoptotic cells evaluated as caspase-3 positive cells. The caspase-3 is a protein effector involved in the apoptosis [19]. Activated caspase-3, as well as caspase-6 and caspase-7, may cleave multiple structural and regulatory proteins, which are critical for cell survival and maintenance [18]. However, caspase-3 is considered the most important executioner caspase and it is involved in both intrinsic and extrinsic apoptotic pathways [20]. Alteration in both mitochondrial function and structure induce the release of mediators such as BCL-2 family proteins that amplify the caspase-3 activity inducing the intrinsic way of apoptosis [21]. Thus, the expression of caspase-3 in breast cells can be related to the occurrence of intrinsic apoptosis. Data of both ki67 and caspase-3 despite in disagreement can be related to the same processes involved in breast cancer progression. Indeed, as demonstrated by Ryoo and Bergmann [22] the caspase-3 related apoptotic phenomenon may be related to cancer progression due to the communication between apoptotic cells and surrounding ones. This biological process physiologically occurs during the embryogenesis, where proapoptotic proteins-mostly caspases-can induce proliferation of neighboring surviving cells to replace dying cells. It is demonstrated that the deregulation of this process in cancer tissues could sustain the tumour proliferation and progress. Thus, only apparently our *in vivo* data seems conflicting. The association of sestamibi uptake with both proliferation index and apoptosis could be considered an instrumental/clinical manifestation of patho-physiological mechanisms commonly involved in breast cancer development. However, a molecular characterization of the events associated to the accumulation of sestamibi into the mitochondria could open new clinical perspectives in the management of breast cancer cells. In fact, the regulation of the mitochondria related apoptotic process is currently one of the main targets of the anti-cancer therapies.

In this context, preliminary data of Erba and colleagues suggested that the sestamibi uptake correlated with the apoptosis level in breast cancer tissues following chemotherapeutic treatment [23].

In this study, the biological link between sestamibi uptake and the apoptotic phenomenon has been investigated *in vitro* by using BT-474 breast cancer cell lines. In particular, three different concentrations of ^{99m}Tc-sestamibi (10 µg/ml, 1 µg/ml, 0.1 µg/ml) have been used to investigate the response of breast cancer cells to the sestamibi uptake. As concern the proliferation index, a significant decrease was observed in breast cancer cells incubated with high concentration of ^{99m}Tc-sestamibi (10 µg/ml). It is important to note that both in controls and at lower concentration of ^{99m}Tc-sestamibi (0.1 µg/ml) no decrease in proliferation index was observed after 120h. Excluding the experiment with high concentration of ^{99m}Tc-sestamibi (10 µg/ml), *in vitro* data confirmed the uptake of sestamibi in breast cancer cells during the proliferation phase. Amazingly, *in vitro*

investigations showed a significant increase in caspase-3 positive cells in cultures incubated with 10 μ g/ml 99mTc-sestamibi. In particular, after 72h the presence of sestamibi in cell medium induced a complete inversion between the proliferation index and apoptosis. In fact, at this time point the number of caspase-3 positive cells was greater than those positive to the Ki67 markers. From morphological point of view, these cell cultures showed numerous death cells characterized by typical apoptotic sign such as pyknosis, or karyopyknosis. In addition, transmission electron microscopy investigations allowed to evaluate the ultrastructure of breast cancer cells incubated with 99mTc-sestamibi demonstrating a progressive increase of the presence of apoptotic alterations. These damages were associated to both 99mTc-sestamibi concentration and incubation time. Remarkably, EDX investigations also showed the presence of technetium into the cytoplasm of apoptotic cells.

All together data of this study suggest that, in the early phases, sestamibi uptake occurs mainly in breast cancer cells with high proliferation index since the high metabolic cell activity and the subsequent increase in the mitochondrial membrane potential. Then, the accumulation of sestamibi into the breast cancer cell mitochondria can induce an alteration of the normal homeostasis thus triggering the apoptotic event.

5. Conclusions

For the first time, this study reported ex vivo and in vitro data about the correlation between sestamibi uptake and apoptosis suggesting the possible role of sestamibi in the regulation of patho-physiological process involved in breast cancer. The evidence of the accumulation of sestamibi into the breast cancer cells and the subsequent mitochondrial damages can open new clinical perspectives in the use of this radiopharmaceutical in both diagnosis and cure of breast cancers. If confirmed by further ex vivo and in vitro studies, the capability of sestamibi to induce the apoptosis of breast cancer cells can lay the scientific rationale for considering this molecule as a theragnostic agent. Indeed, different concentrations of 99mTc-sestamibi could be used for the detection of cancer lesions with high proliferation index or to stimulate the apoptosis, thus countering cancer growth. Lastly. These investigations further highlight the fundamental cooperation between nuclear medicine, and in general imaging diagnostic, and the pathology in both research and diagnostic applications [24,25].

Author Contributions: Conceptualization: N.U., M.S., and O.S.; Methodology: M.S., R.B., E.B., and A.M.; Formal analysis R.B., and E.B.; Data curation: A.M., N.U. and O.S.; Writing—original draft preparation: N.U., M.S., and O.S.; Writing—review and editing: R.B., E.B. and A.M.; Supervision, O.S. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy concerns.

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