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Fagner Santos do Carmo

Estudo e desenvolvimento de nanorradiofármaco à base de aptâmero anti-MUC1 para diagnóstico precoce de processos carcinogênicos de mama

Rio de Janeiro

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Tese apresentada, como requisito parcial para obtenção do título de Doutor, ao Programa de Pós-Graduação em Biociências, da Universidade do Estado do Rio de Janeiro.

Orientador: Prof. Dr. Ralph Santos-Oliveira

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2017

DEDICATÓRIA

Dedico este trabalho à minha família, à minha noiva e aos amigos que foram simplesmente essenciais para a conclusão deste trabalho.

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Nelson Mandela

RESUMO

DO CARMO, Fagner Santos. *Estudo e desenvolvimento de nanorradiofármaco à base de aptâmero anti-MUC1 para diagnóstico precoce de processos carcinogênicos de mama*. 2017. 74 f. Tese (Doutorado em Biociências) – Instituto de Biologia Roberto Alcântara Gomes, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, 2017.

O câncer de mama é o tipo de neoplasia que mais causa mortes entre as mulheres em todo o mundo. Um dos entraves mais prevalentes na área oncológica que correspondem a alta taxa de morbidade é a ausência de detecção precoce dos processos carcinogênicos, especialmente em casos de câncer de mama triplo negativo (TNBC). A não descoberta de um câncer abaixo do nível detectável por testes de rastreios atuais, inclusive por meio de amostras sanguíneas, aponta para a real necessidade de ferramentas tecnológicas inovadoras, que sejam direcionadas especificamente ao microambiente tumoral, com o mínimo de perturbações aos tecidos saudáveis. A utilização de aptâmeros é uma evolução da terapia alvo-dirigida, apresentando considerável nível de sensibilidade a substratos particulares, como a mucina 1 (MUC1). Neste estudo, o aptâmero anti-MUC1 foi o direcionador utilizado em nanopartículas poliméricas radioativas para geração de imagem de TNBCs. Assim, as NPs de poli (ácido láctico-co-glicólico) carregadas com o aptâmero anti-MUC1 e radiomarcadas com tecnetium-99m foram utilizadas para realização de estudo de biodistribuição e imagem de TNBC. Os resultados confirmaram que as NPs foram obtidas com sucesso, com um tamanho médio de 262 nm, de acordo com os dados dinâmicos de dispersão da luz. O teste de biodistribuição em modelos animais induzidos com TNBC mostrou que, embora tenha havido uma alta captura pelo intestino (30%), o sistema de entrega de fármaco (DDS) desenvolvido apresentou uma alta absorção no tumor (5%) e excelentes propriedades de imagem in vivo, corroborando a possibilidade de uso desse DDS como radiofármaco para imagem em TNBC.

Palavras-chave: Aptâmero. Câncer de mama. Imagem. Medicina nuclear. Radiofarmácia.

ABSTRACT

DO CARMO, Fagner Santos. *Estudo e desenvolvimento de nanorradiofármaco à base de aptâmero anti-MUC1 para diagnóstico precoce de processos carcinogênicos de mama*. 2017. 74 f. Tese (Doutorado em Biociências) – Instituto de Biologia Roberto Alcântara Gomes, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, 2017.

Breast cancer and the type of neoplasia that causes more deaths among women around the world. One of the most prevalent barriers in the oncology area that corresponds to the high morbidity rate is the absence of early detection of the carcinogenic processes, especially in cases of triple negative breast cancer. Non-discovery of a cancer below the level detectable by current screening tests, including by means of blood samples, points to the real need for innovative technological tools that are specifically targeted to the tumor microenvironment, with minimal disruption to healthy tissues. The use of aptamers is an evolution of target-directed therapy, presenting a considerable level of sensitivity to particular substrates, such as mucin 1 (MUC1). In this study, the anti-MUC1 aptamer was used as a drug delivery system (DDS) for a radioactive polymeric nanoparticle (NP) in the imaging of TNBCs. Thus, poly(lactic-co-glycolic acid) NPs loaded with the anti-MUC1 aptamer and labeled with technetium-99m were used for a biodistribution study and imaging of TNBC. The results confirmed that the NP was successfully obtained, with a mean size of 262 nm, according to the dynamic light scattering data. The biodistribution assay in induced animal models with TNBC showed that although there was a high capture by intestine (30%), the DDS developed had a high tumor uptake (5%) and with great in vivo imaging properties, corroborating the possibility of use of this DDS as an imaging drug for TNBC.

Keywords: Aptamer. Breast cancer. Imaging, nuclear medicine. Rradiopharmacy.

LISTA DE ILUSTRAÇÕES

LISTA DE ABREVIATURAS E SIGLAS

BRCA	*BReast CAncer type* – Tipo de Câncer de Mama
BCT	*Breast Conserving Therapy*
BCS	*Breast Conserving Surgery*
CK	*Cytokeratins*
CNEN	Comissão Nacional de Energia Nuclear
CT	*Computerized Tomography*
CSCs	Cancer Stem Cells
DCIS	*Ductal carcinoma in situ*
DCM	*Dichloromethane* – Diclorometano
DDS	*Drug Delivery System* – Sistema de entrega de droga
DLS	*Dynamic Light Scattering*
DNA	*Deoxyribonucleic Acid* – Ácido Desoxirribonucleico
EGRF	Receptor do Fator de Crescimento Epidérmico
ER	*Estrogen receptor*
HER2	*Human Epidermal growth factor Receptor-type 2* - Fator de Crescimento Epidérmico Humano
IEN	*Nuclear Energy Research Institute* – Instituto de Engenharia Nuclear
IGF-1	*Insulin Growth Factor 1* – Fator de crescimento Insulina-1
IMPC	*International Mouse Phenotyping Consortium*
IMRT	Instituto de Pesquisas Energéticas e Nucleares
IPEN	*Intensity Modulated Radiation Therapy*
JUN	*Gene* JUN
MBC	*Metaplastic Breast Cancer*
MPD	*Mammary Paget Disease*
MPS	*Mononuclear phagocytic system* – Sistema Fagocitário Mononuclear
MRI	*Magnetic Resonance Imaging*
mTOR	*Mammalian Target of Rapamycin*
MUC1	*Mucin 1*
MYC	MYC *gene*
NACT	*Neoadjuvant Chemotherapy*

NP	*Nanoparticle* – Nanopartícula
NPs	*Nanoparticles* – Nanopartículas
OMS	Organização Mundial da Saúde
O/W/O	*Oil-in-water-in-oi l* – óleo-água-óleo
PARP	poly ADP-ribose polymerase
PET	*Positron Emission Tomography*
PLGA	*poly (lactic acid-co-L-acid)*
PMRT	*Post Mastectomy Radiation*
PR	*Progesterone receptor*
PTEN	*Phosphatase and Tensin Homolog*
PVA	*polyvinyl alcohol* – álcool polivinílico
RAS	Gene humano – oncogene (*RAt Sarcoma vírus* – vírus do sarcoma de rato)
RNA	*Ribonucleic acid* – Ácido Ribonucléico
ROIs	*Regions of interest* – Região de interesse
RPMI	Meio para Cariótipo
sc	*Subcutaneous* – subcutâneo
SELEX	*Systematic Evolution of Ligands by Exponential Enrichment*
SPECT	*Single-photon Emission Computed Tomography* – Tomografia Computadorizada por Emissão de Fóton Único
TNBC	*Triple-negative breast câncer* - Câncer de mama triplo negativo
TZB	Trastuzumab
UK	*United Kingdom* – Reino Unido
US	*Ultrasonography*
USA	*The United States of America* – Estados Unidos da América

LISTA DE SÍMBOLOS

3D-CRT	*Three-Dimensional Conformal Radiation Therapy*
5-FU	5-fluorouracil
16q20	Cromossomo 16 (16q20)
17p13	Cromossomo 17 (17p13)
^{99m}Tc	*Technetium*-99m – Tecnécio^{99m}
BCL2	BCL2 *gene*
CDK4	*Cyclin Dependent Kinase* 4
CH_2Cl_2	Cloreto Estanoso
cm	Centímetro
FOXA1	FOXA1*gene*
GATA-3	*Proteína codificada pelo gene GATA-3 em humanos*
kDa	Quilodalton
keV	Quilo elétron-volt
kHz	Quilohertz
Ki67	Proteína nuclear
MDA-MB-231	Tipo de linhagem celular de mama humana
mCi	Milicurie
MBq	Megabecquerel
MIBI	2-metoxi-2-isobutil isonitrila
MUC 1	Mucina 1
µCi	Microcurie
µL	Microlitro
mL	Mililitro
nm	Nanômetro
p53	Proteína citoplasmática, de massa molecular 53 kDa
P.A.	Para Análise
RAF	RAF *gene*
$SnCl_2$	Cloreto Estanoso
TBX3	TBX3 *gene*
uCi	Milicurie

W	Watts
%	Porcentagem
Σ	Somatório
±	Mais ou menos

SUMÁRIO

INTRODUÇÃO

O câncer de mama é a principal causa de morte relacionada ao câncer entre as mulheres em todo o mundo (FERLAY et al., 2015; SIEGEL et al. 2015). As taxas de sobrevivência observadas em países desenvolvidos e em desenvolvimento, quando há acesso à tratamentos eficazes e diagnósticos precoces, são elevadas, quando comparadas a outros tipos de câncer (ALTEKRUSE et al., 2007; GLOBOCAN, 2012; KNOBF et al., 2012; YOULDEN et al., 2012; KOCH et al., 2013). No entanto, estudos mais recentes relacionam um aumento do número de mortalidade à regiões menos favorecidas socioeconomicamente (DANFORTH, 2013; GOSS et al., 2013; JUSTO et al., 2013; TORRE et al., 2015; TORRE et al., 2016).

Segundo os dados do Atlas de mortalidade por câncer no Brasil, entre os anos de 2004 a 2014 o câncer de mama causou cerca de 134.021 óbitos, concentrando mais que 50% dos casos entre as idades de 40 anos a 69 (BRASIL, 2016). A estimativa é que o câncer de mama sozinho responderá por 29% de todos os novos diagnósticos de câncer em mulheres no mundo (SIEGEL, MILLER and JEMAL, 2016) e de acordo com Howlader et al. (2014) 12% da população feminina desenvolverá câncer de mama durante sua vida. Para os anos de 2016 e 2017, no Brasil, espera-se a incidência de 420 mil casos novos de câncer, sendo a neoplasia mamária mais frequente nas regiões Sul, Sudeste e Centro-Oeste (BRASIL, 2015).

O câncer de mama é bem divergente com relação à clínica e à morfologia. Sendo classificado em mais de 20 subtipos tumorais diferentes pela Organização Mundial de Saúde (OMS) (BRASIL, 2015). Alguns trabalhos epidemiológicos o associam ao avanço da idade biológica e, de alguma forma, relacionam ao estilo de vida, ao ambiente e a dieta, sem mencionar fatores genéticos, alta densidade do tecido mamário, etnia, hormônios e à fatores relacionados à vida reprodutiva da mulher, e exposição à radiação ionizante. (LAND et al., 2003; HOLMES et al., 2005; CHLEBOWSKI et al., 2006; DALEY et al., 2007; PIERCE et al., 2007; MARTIN andBOYD, 2008; NASIR et al., 2009; FEJERMAN et al., 2010; BOYD et al., 2011; KUSHI et al., 2012; NAROD, 2012; BAE et al., 2016; CONROY et al., 2016; RUNOWICZ, et al., 2016).

A ocorrência de casos de câncer de mama em pacientes com idade inferior aos 50 anos é bem alta, particularmente os agressivos, de crescimento rápido, com presença de mutações nos genes BRCA 1 e BRAC 2 (EASTON, 1999; PAL et al., 2005; CAMPEAU, FOULKES and TISCHKOWITZ, 2008; NAROD, 2012; VILLARREAL-GARZA et al., 2013; REYNA

and LEE, 2014). O que sugere a forte contribuição dos hormônios femininos no desenvolvimento da doença (FERLAY et al., 2015).

O câncer de mama triplo negativo (TNBC - Triple-negative Breast Cancer) ocorre em 10% a 20% de todos os cânceres de mama (MORRIS, et al., 2007; DIETZE et al., 2015), afetando , proponderantemnete, pacientes mais jovens (ELEY et al., 1994; FURBERG et al., 2001; PORTER et al., 2004; BAUER et al., 2007; MORRIS, et al., 2007; LARA-MEDINA, et al. 2011; HUDIS AND GIANNI, 2011; LIEDTKE et al., 2013). É mais prevalente em mulheres africanas (ELEY et al., 1994; FURBERG et al., 2001; PORTER et al., 2004; BAUER et al., 2007; MORRIS, et al., 2007; BADVE et al., 2010; AMIRIKIA et al., 2011; AGBOOLA et al., 2013; DANFORTH,. 2013; PARISE and CAGGIANO, 2015; DIETZE et al., 2015; MOUH et al., 2016) e em hispânicas (ELEY et al., 1994; BAUER et al., 2007; BANEGAS et al., 2014; SINESHAW et al., 2014). Muitos autores correlacionam a incidência do TNBC a mulheres pertencentes a grupos socioeconômicos mais baixos (ELEY et al., 1994; VONA-DAVIS and ROSE, 2009; ANDAYA et al., 2012; PARISE and CAGGIANO, 2015; SINESHAW et al., 2014; AKINYEMIJU et al., 2015; DIETZE et al., 2015; AUGUSTE et al., 2017).

1 **OBJETIVOS**

1.1 **Objetivo geral**

Desenvolver e caracterizar nanopartículas de PLGA modificadas com aptâmero anti MUC 1 e radiomarcá-las com Tecnécio-99m, para obtenção de agente diagnóstico de tumores de mama com superexpressão do gene mucina 1.

1.2 **Objetivos específicos**

- Produção de nanopartículas de PLGA;
- Modificação das nanopartículas de PLGA com aptâmero anti MUC 1;
- Marcação das nanopartículas de PLGA vazias e modificadas com aptâmero Anti MUC 1com o radionuclídeo Tecnécio-99m;
- Avaliação da biodistribuição das nanpoartículas de PLGA vazias e modificadas com Anti MUC1 marcadas com Tecnécio-99m em camundongos fêmeas BALB/c saudáveis e com modelo xenográfico de câncer de mama desenvolvido.

2 METODOLOGIA CIENTÍFICA

2.1 Anti-Muc1 Nano-Aptamers for Triple-Negative Breast Cancer Imaging by Single-Photon Emission Computed Tomography in Inducted Animals: Initial Considerations (Artigo científico publicado)

Abstract: The early and specific detection of tumors remains a barrier in oncology, especially in cases such as the triple-negative breast cancer (TNBC). To address this gap, aptamers have found an important application in the recognition of tumor biomarkers such as mucin 1 (MUC1). However, there are still some difficulties in the use of aptamer, as their rapid biological clearance makes their use as drugs limited. In this study, the anti-MUC1 aptamer was used as a drug delivery system (DDS) for a radioactive polymeric nanoparticle (NP) in the imaging of TNBCs. Thus, poly(lactic-co-glycolic acid) NPs loaded with the anti-MUC1 aptamer and labeled with technetium-99m were used for a biodistribution study and imaging of TNBC. The results confirmed that the NP was successfully obtained, with a mean size of 262 nm, according to the dynamic light scattering data. The biodistribution assay in induced animal models with TNBC showed that although there was a high capture by intestine (30%), the DDS developed had a high tumor uptake (5%) and with great in vivo imaging properties, corroborating the possibility of use of this DDS as an imaging drug for TNBC.

Keywords: aptamer, cancer control, imaging, nuclear medicine, radiopharmacy.

INTRODUCTION

Breast cancer is one of the most common cancers and afflicts thousands of women worldwide. The incidence rates vary greatly worldwide, from 19.3 per 100,000 women in Eastern Africa to 89.7 per 100,000 women in Western Europe. In most of the developing regions, the incidence rates are ,40 per 100,000 and it is estimated that over 508,000 women died in 2011 because of breast cancer, worldwide.1,2

Triple-negative breast cancer (TNBC) is characterized by a low expression of the estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2. TNBC tends to behave more aggressively than the other breast cancer subtypes (has rapid growth and higher chance of recurrence and metastasis). 3,4 TNBC accounts for ~20% of all breast cancers and is more frequently diagnosed in women aged .40 years and particularly of African descendancy.5–7 There is strong evidence that links TNBC with the mutation of

BRCA gene.8 The diagnosis of TNBC is currently performed using a microarray technique.9,10 However, both diagnosis and treatment remain a clinical challenge.

In recent years, TNBC has been linked to a specific immunologic response originating from the large expression of the mucin 1 (MUC1) gene. MUC 1 belongs to the family of genes that encodes transmembrane glycoproteins type I, with high molecular weight, 11,12 and is present ubiquitously in the apical surface of glandular epithelial cells, including gastrointestinal, respiratory, urinary, and reproductive tract.12 Overexpression of MUC1 has been identified as a marker of malignancy in several primary tumors, such as breast, ovarian, colon, lung, gastric, pancreatic, and prostate cancers.11,13–17 Normal tissue and tumor have the same amino acid sequence, but only distinguished by MUC 1 overexpression by cancer cells and its aberrant glycosylation pattern 16.

MUC1 has been the target for many therapeutic approaches, including antibodies, vaccine therapies, and aptamers.18,19 Aptamers are synthetic oligonucleotides, such as ribonucleic acid (RNA) and single-stranded deoxyribonucleic acid, which can bind to their targets with high affinity and specificity because of their specific secondary and tertiary structures.20 Compared with antibodies, they offer a great potential in targeting tumor markers like MUC1 because of their small size, lack of immunogenicity, and superior tumor penetration.21 In addition, they have been capable of detecting circulating MUC1 in sandwich enzyme-linked immunosorbent assays, improving current detection limits,19 and have been extensively studied as radiopharmaceuticals for their potential in gamma-camera and single-photon emission computed tomography (SPECT) imaging.21–23 Furthermore, they have been shown to be particularly promising agents in photodynamic therapy as phototoxic agents 24 and delivery agents of standard chemotherapy such as doxorubicin.25 Finally, the MUC1 aptamers have been successfully used in nanoparticle (NP) formulation, described both from our own group26 for silica NPs and from a different group for liposomal formulation in conjunction with paclitaxel.27 The properties of high affinity and specificity toward their targets make aptamers the molecules of choice to be used as delivery agents. Aptamers can be designed as targeting ligands, 20 with their properties modified at demand.

The use of nanotechnology in cancer treatment and diagnosis is rapidly evolving. In recent years, many research groups have been devoting their efforts to the development of NPs to interrogate specific molecular targets (imaging probes) and to deliver systemic radiotherapy to those targets, while minimizing the toxicity to normal cells, following what has been called the “magic bullet” concept. In this fashion, NPs conjugated with unstable radioisotopes for positron emission tomography and SPECT imaging have the highest

potential for successful imaging because of their inherited high sensitivity. The same NPs can be conjugated with beta-minus- or alpha-particle-emitting radioisotopes for targeted radiotherapy. 28–32 In this study, unique nano-radiopharmaceuticals with polymeric NPs of the anti-MUC-1 aptamer labeled with technetium-99m (99mTc) were developed for the early diagnosis of MUC1 overexpression in TNBC. These novel imaging compounds have the potential to play an important role in the development of improved imaging strategies for TNBC.

Aptamers

The aptamers used in this study have been those previously described by Ferreira et al18 and used in various studies as radiopharmaceuticals alone or in multimeric and pegylated complexes.21–23 These aptamers have been selected against the APDTRPAPG synthetic peptide of the MUC1 tandem repeat sequence using traditional SELEX approaches. The structure of this anti-MUC1 aptamer has also been studied by nuclear magnetic resonance 33 and deposited in Protein Data Bank.

Nanoparticles

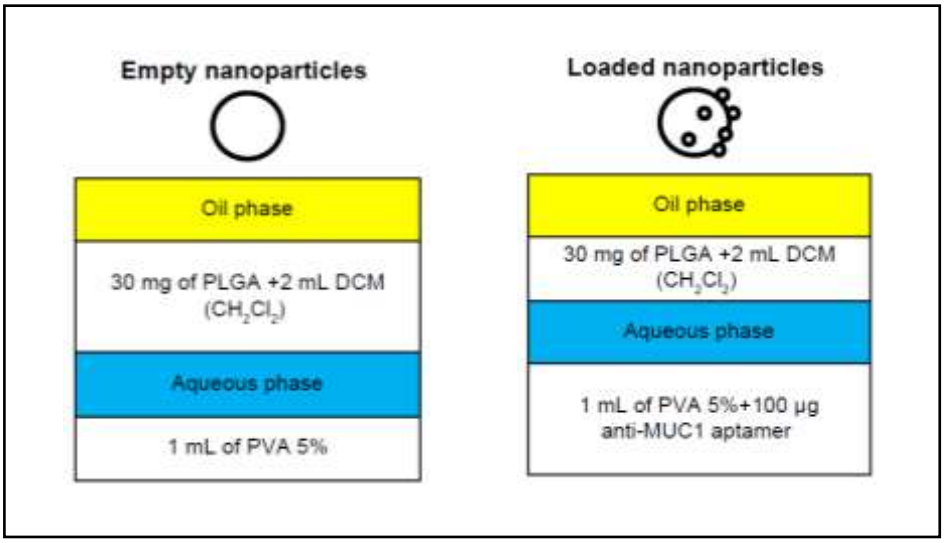
Two NPs were prepared: the first one empty (no anti-MUC1) and the second one with anti-MUC1 aptamer.

Empty NP

Thirty milligrams of poly (lactic acid-co-L-acid) (PLGA) was dissolved in 2 mL of CH_2Cl_2 (solution A). Then 1 mL of solution of polyvinyl alcohol (PVA) 5% was dripped over solution A and then ultrasonicated by 2 cycles of 30 seconds in a potency of 55 W, forming an emulsion (solution B). The solution B was dropped into 40 mL of a solution of PVA 1% and ultrasonicated by 2 cycles of 30 seconds in a potency of 55 W. The final product was a PLGA emulsion system oil-in-water-in-oil (O/W/O). This final solution was dried under low pressure at room temperature for 40 minutes to eliminate the excess of dichloromethane.

Loaded NP with aptamer anti-MUC1

Thirty milligrams of PLGA was dissolved in 2 mL of CH_2Cl_2 (solution A). Then 1 mL of a solution of PVA 5% and 100 µg of anti-MUC-1 aptamer was dripped over the solution A and then ultrasonicated in 2 cycles of 30 seconds with a potency of 55 W, forming an emulsion (solution B). The solution B was dropped into 40 mL of a solution of PVA 1% and ultrasonicated in 2 cycles of 30 seconds with a potency of 55 W. The final product was a PLGA emulsion system O/W/O. This final solution was dried under lower pressure at room temperature for 40 minutes to eliminate the excess of dichloromethane (Scheme 1).



Scheme I Schematic process of the formation of nanoparticles.

Source: Prepared by the author.

Abbreviations: DCM, dichloromethane; MUC1, mucin 1; PLGA, poly(lactic-*co*-glycolic acid); PVA, polyvinyl alcohol.

Size determination by dynamic light scattering

NP size distribution, mean size, and polydispersity index of the NPs were determined by dynamic light scattering (DLS) using the equipment Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK). The measurements were performed in triplicate at 25°C, and the laser incidence angle in relation to the sample was 173° using a 12 mm2 quartz cuvette. The mean ± standard deviation was assessed.

Labeling with 99mTc-nano-radiopharmaceuticals

The method used was the direct labeling process as described by Sá et al. 26 and Albernaz et al. 34 The labeling process used 150 µL of NPs (empty and loaded), which were incubated with SnCl2 solution (30 µL/mL) (Sigma-Aldrich, St Louis, MO, USA) for 20 minutes at room temperature. Then this solution was incubated with 2 mCi (~300 µL) of 99mTc (Instituto de Pesquisas Energeticas e Nucleares [IPEN/CNEN]) for 10 minutes, which labeled the NPs with 99mTc.

To characterize the labeled NPs (empty and loaded), paper chromatography was carried out using Whatman No 1 paper. The paper chromatography was performed using 2 µL of the labeled NP in acetone (Sigma-Aldrich) as mobile phase. The radioactivity of the strips was verified in a gamma counter (Perkin Elmer Wizard® 2470, Perkin Elmer, Shelton, CT, USA). To confirm the efficacy of the labeling process of the NP (empty and loaded), paper chromatography was performed at 8 hours (Table 1).

Table 1 Efficacy of the labeling process with technetium-99m of the PLGA nanoparticles

Nanoparticle type	Efficacy of labeling at 8 hours
PLGA–nanoparticle (empty)	99.76±0.8
PLGA–aptamer–nanoparticle	99.83±0.7

Source: Prepared by the author.

Abbreviation: PLGA, poly(lactic-*co*-glycolic acid).

In vivo analysis

Tumor xenograft models

MDA-MB-231 cells (American Type Culture Collection, Manassas, VALLC) were cultured in RPMI (Thermo Fisher Scientific, MA USA) supplemented with 10% of fetal

bovine serum (Gibco) and 50 µg/mL of gentamicin (Gibco). Mycoplasma contamination in cultured cells was excluded using Lonza Mycoplasma Detection Kit.

Tumors were established by subcutaneous (sc) injection of 2×106 MDA-MB-231 cells at the back of 6-week-old female BALB/c nude mice. The tumor size was monitored for 3 weeks and measured by a caliper. The tumor size before imaging was about 2 cm in diameter. The BALB/c nude mice were bred at the animal facility of the Nuclear Energy Research Institute (IPEN). This study and the animal procedures were approved by the University of Pernabuco Ethics Committee, under the number: 23076020578201327. All animal experiments were done in accordance with the regulations and guidelines of Brazilian Law for animal experiments (Law number 11.794/2008 and Decree 6.899/2009). These mice were observed three times per week for evidence of distress, ascites, paralysis, or excessive weight loss.

Biodistribution studies

Evaluation of the biodistribution of NPs was performed using two groups: 1) control group using empty NPs (n=6) and 2) intervention group using NPs loaded with aptamer (n=6), both labeled with 99mTc. The mice were anesthetized with mixed solution of 10% ketamine and 2% xylazine in a volume of 15 µL and administered intramuscularly (thigh). The nano-radiopharmaceuticals (3.7 MBq in volume of 0.2 mL) were administered retro-orbitally.34 Both groups were killed by asphyxiation using a carbon dioxide gas chamber after 2 hours (120 minutes) of radio-compound administration. Organs (brain, lungs, kidneys, stomach, small and large intestine, bladder, heart, and blood pool) were removed and weighted and the activity in each organ and blood was counted by a gamma counter (Perkin Elmer Wizard 2470). The results were expressed as uCi per organ 26,34.

SPECT imaging

Planar images were obtained at 90 minutes after injection of nano-radiopharmaceuticals (3.7 MBq in volume of 0.2 mL) using a Millenium Gamma Camera (GE Healthcare, Cleveland, OH, USA). Counts were acquired for 5 minutes in a 15% window centered at 140 keV. The images were processed using OsiriXsoftware, and regions of interest

(ROIs) over the tumor were selected for specific analysis. Three induced mice were imaged separately.

Results and discussion

DLS size characterization

Figure 1 shows the mean size and size distribution of the NPs (empty and loaded). According to the distribution profile it is possible to infer that NPs presented a monomodal size distribution, with a mean size of 255 nm for the empty NPs and 262 nm for the aptamer–NPs. These results were not statistically different when comparing the two NPs, indicating that there is a slight increase in size for the NP containing the aptamer on its surface. However, as the aptamer is a small molecule it does not seem to increase greatly the size of the polymer NP (aptamers have a molecular weight ranging from 5 to 15 kDa). Narrow peaks suggested homogeneous systems with sizes near to the mean.

Labeling process

All the PLGA NPs using 1) empty and 2) aptamer-loaded anti-MUC1 were successfully labeled with 99mTc. The average of labeling efficacy was over 97% in all cases.

Tumor xenograft model

After 3 weeks of the sc injection of 2×106 MDA-MB-231 cells the tumor was palpable and visible as in Figure 2.

Biodistribution study

NP loaded with anti-MUC1 aptamer

The biodistribution data (Figure 3) demonstrated that the nano-aptamer has a high hydrophilicity, and for that reason has a high uptake by the kidneys (Σ Kidneys 0.4131 μCi). It means that 48.43% of the nano-radiopharmaceuticals were in the kidneys. This is important for two reasons. First, it demonstrates the renal clearance of the nano-radiopharmaceutical, which is a desirable property. Second, the reabsorption mechanism present in the kidneys will probably help the biodistribution of the NP in the whole body 35,36.

The presence of MUC1 in the intestines (large and small) 37,38 may, in some cases, interfere in the biodistribution of the nano-aptamer labeled with 99mTc, because it is a pan-epithelial mucin. 39–41 In this case, the nano-aptamer labeled with 99mTc showed an uptake of 16.71% in the large intestine (0.13753 μCi) and 19.65% in the small intestine (0.16172 μCi). Furthermore, the size of the nano-aptamer labeled with 99mTc may interfere in the biodistribution, because it may be trapped into the intestinal microvilli because of its higher irrigation. However, as the labeled empty NP had a lower intestine uptake, this seems a less plausible explanation, indicating a potential interaction of the MUC1 aptamer (see the following text and Figure 4).

NPs suffer from the limitation of rapid clearance by the mononuclear phagocytic system (MPS) located primarily in the liver and spleen, thereby limiting the dose available for the disease site. 42,43 In this particular case the spleen uptake was very low (0.01504 μCi) representing 1.82% of the total dose. The use of PLGA NP has demonstrated lower uptake by the spleen, and, thus, an advantage in the use of this NP. In addition, the presence in the liver was also low. The total amount was 0.11917 μCi, representing 14.48% of the total nano-aptamer labeled with 99mTc, less than the uptake by the intestines, also representing an advantage of this NP system.

The uptake by the lesion (tumor) showed a value of 0.0499 μCi (5%) of the nano-aptamer labeled with 99mTc, which supports the use of the nano-aptamer as an imaging agent, also corroborating the imaging results. Is is important to notice that the uptake in the brain is negligible.

Empty NP

The results of the biodistribution of the empty NP (Figure 4) showed renal clearance with a total of 77% of the empty NP uptake by the kidneys (Σ Kidneys 1.9627 μCi).

The presence of MUC1 in the intestines did not interfere in the biodistribution of the empty NP, as expected. The values in the large intestine (0.7%) and small intestine (1.66%) were quite different from that observed in the loaded NP, showing that the presence of the aptamer in the NP was continuous throughout the biodistribution test.

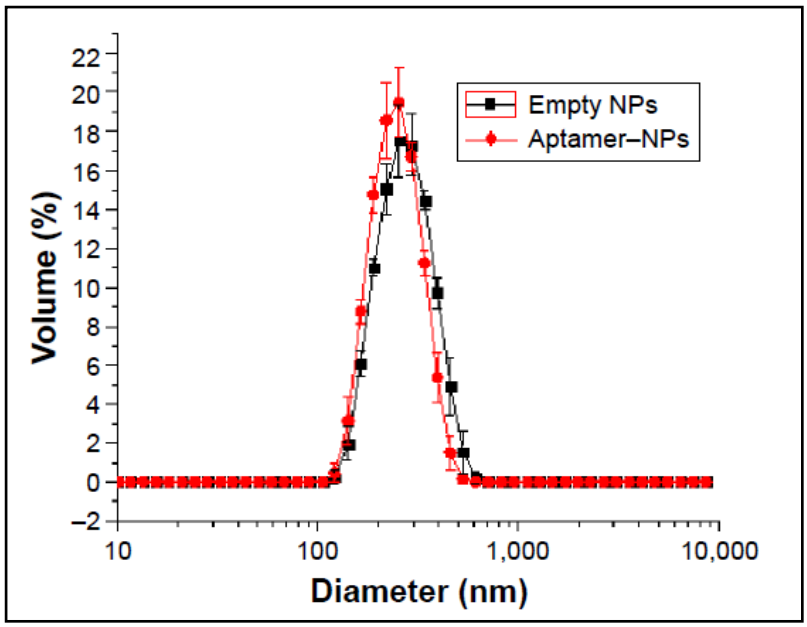


Figure 1 Mean size and size distribution of the nanoparticles.

Notes: The empty NPs are in black and the aptamer–NPs are in red. Analyses were performed at 25°C after preparation. Error bars indicate standard deviation for the triplicates.

Abbreviation: NPs, nanoparticles.

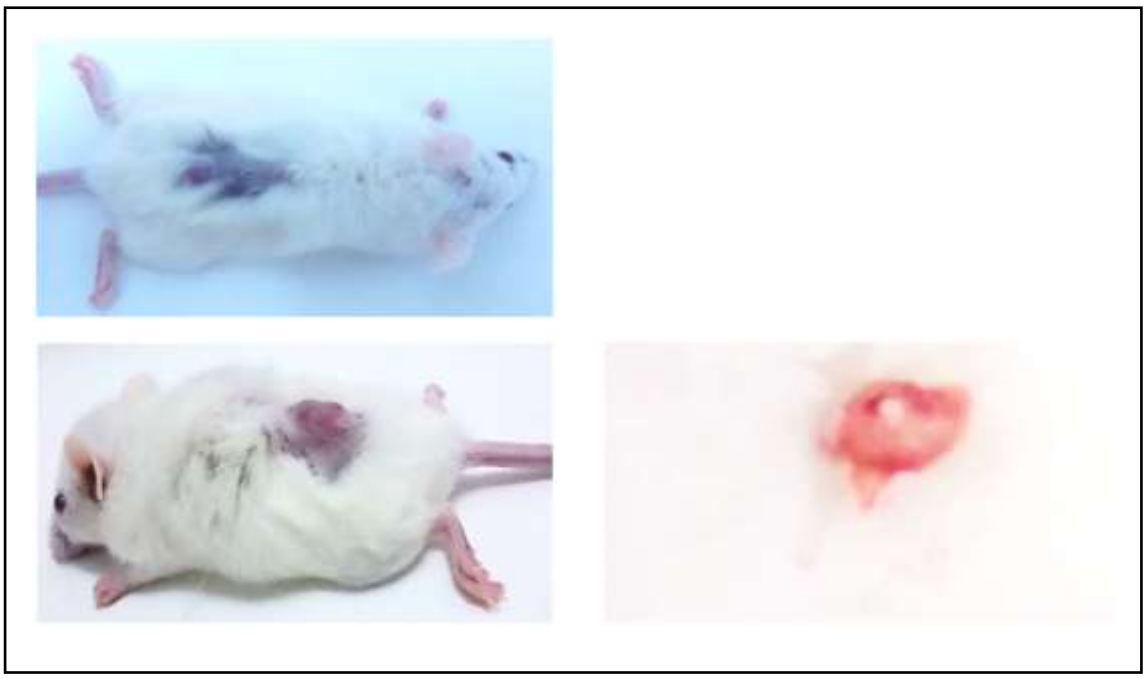


Figure 2 Tumor growth after 3 weeks of injection of 2×106 MDA-MB-231 cells in the back dorsal region of mice.

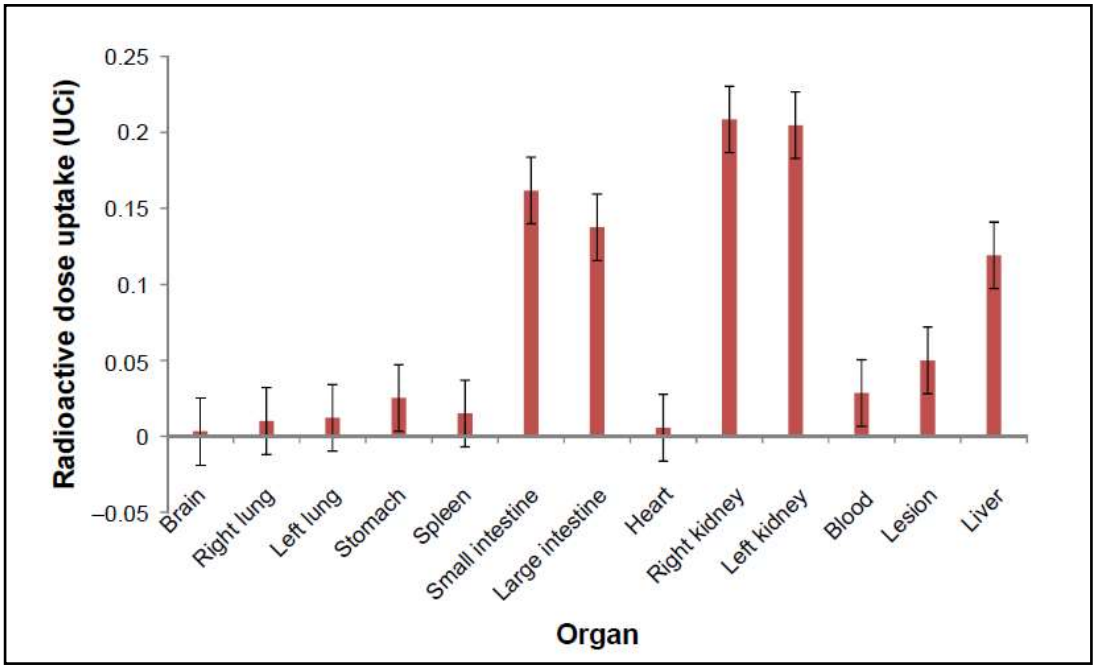


Figure 3 Biodistribution of loaded nanoparticle in induced mice.

Note: Error bars were calculated using the mean ± standard deviation.

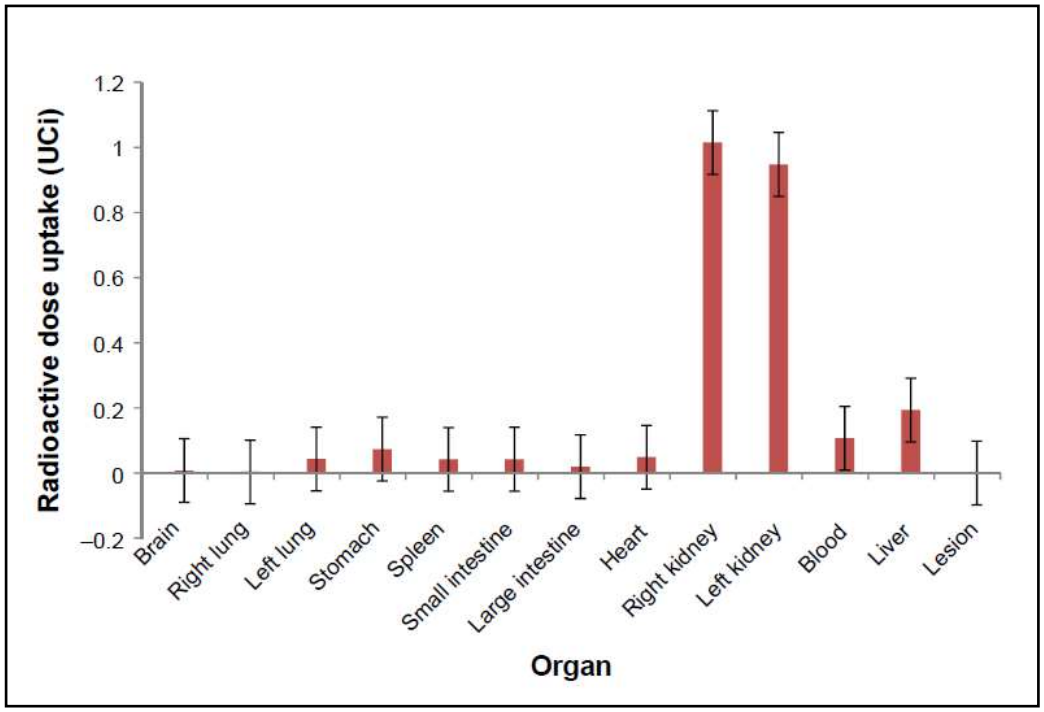


Figure 4 Biodistribution of empty nanoparticle in induced mice.

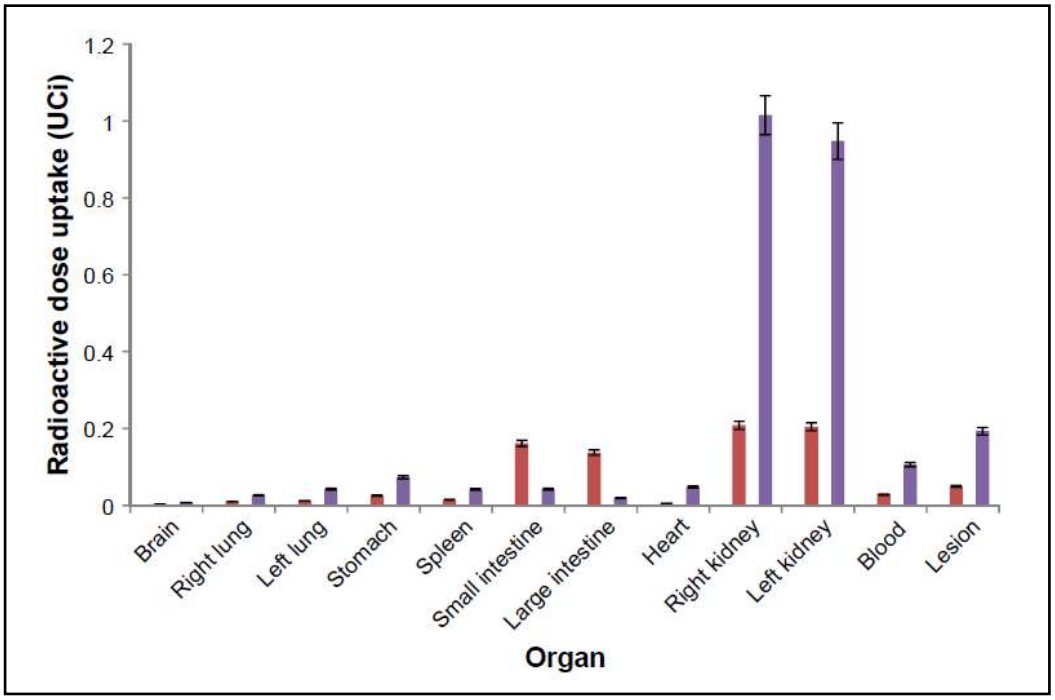


Figure 5 Comparison of biodistribution of empty (purple) and loaded (red) nanoparticle in inducted mice.

The small amount of NP in the stomach (0.07351 µCi) demonstrated no formation of colloid with the 99mTc. It is important to note the presence of higher amount in spleen (0.04189 µCi; 1.6%) and liver (0.1933 µCi; 7.6%), showing that the empty NP is taken up by the MPS, although in low concentrations. These data are important because, when comparing with the loaded NP, the uptake by the spleen is the same; however, the uptake by the liver is twice as much in the loaded NP. This means that the presence of the anti-MUC1 aptamer in the NP stimulates the MPS, especially in the liver. There was no uptake by the empty NP by the lesion. The amounts in brain, blood, and other organs are negligible.

To better understand the results of both graphics, they are shown together (Figure 5). It is possible to observe the higher uptake by the lesion and the better renal clearance by the drug delivery system.

SPEC T imaging

In the SPECT (Figure 6) it is possible to observe the higher uptake in the lesion area. The ROI showed that the increased uptake region (yellow) was because of the biodirection of the anti-Muc1 aptamer NP accumulation. It is also possible to observe the kidney uptake in the image (lower region), corroborating the biodistribution result. The increased uptake region is because of the retro-orbital injection and is the remaining radioactivity in the injection site. The imaging findings, together with the results from the biodistribution assay, confirm the possible use of this NP as an imaging agent.

Conclusion

The results confirmed that the nano-radiopharmaceuticals based on the anti-MUC1 aptamer could be used as an imaging agent for TNBC. The results of the biodistribution study aligned with the SPECT imaging results, suggesting that this drug delivery system may represent a safe and alternative agent for the TNBC. The renal clearance and the low uptake by the liver and spleen suggest its potential application in human beings.

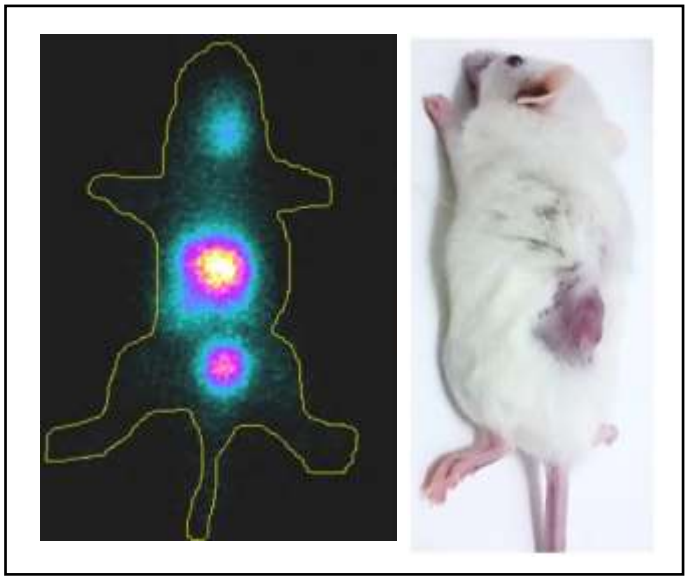


Figure 6 Scintigraphy of anti-MUC1 nano-aptamer in animals induced with triplenegative breast cancer.

Abbreviation: MUC1, mucin 1.

Disclosure

The authors report no conflicts of interest in this work.

References

1. GLOBOCAN 2012: cancer incidence and mortality worldwide in 2012 – IARC; 2012. Available from: http://globocan.iarc.fr/Pages/fact_sheets_population.aspx. Accessed October 10, 2016.

2. Chappuis P, Rosenblatt J, Foulkes W. The influence of familial and hereditary factors on the prognosis of breast cancer. Ann Oncol. 1999;10(10):1163–1170.

3. Sorlie T. Molecular portraits of breast cancer: tumors subtypes as distinct disease entities. Eur J Cancer. 2004;40(18):2667–2675.

4. Jemal A, Center MM, Ward E, Thun MJ. Cancer occurrence. Methods Mol Biol. 2009;471:3–29.

5. Carey LA, Perou CM, Livasy CA. Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. JAMA. 2006;295(21):2492–502.

6. Reis-Filho JS, Westbury C, Pierga JY. The impact of expression, profiling on prognostic and predictive testing in breast cancer. J Clin Pathol. 2006;59(3):225–231.

7. DeSantis C, Ma J, Bryan L, Jemal A. Breast cancer statistics, 2013. CA Cancer J Clin. 2014;64(1):52–62.

8. Chabalier C, Lamare C, Racca C, Privat M, Valette A, Larminat F. BRCA1 down regulation leads to premature inactivation of spindle checkpoint and confers paclitaxel resistance. Cell Cycle. 2006;5(9):1001–1007.

9. Jeffrey SS, Fero MJ, Børresen-Dale AL, Botstein D. Expression array technology in the diagnosis and treatment of breast cancer. Mol Interv. 2002;2(2):101–109.

10. Dong M, How T, Kirkbride KC, et al. The type IIITGF-β receptor suppresses breast cancer progression. J Clin Invest. 2007;117:206–217.

11. Gender SJ, Spicer AP. Epithelial mucin genes. Annu Rev Physiol. 1995;57:607–634.

12. Gender SJ. MUC1, the renaissance molecule. J Mammary Gland Biol Neoplasia. 2001;6(3):339–353.

13. Girling A, Bartkova J, Burchell J, Gendler S, Gillett C, Taylor-Papadimitriou J. A core protein epitope of the polymorphic epithelial mucin detected by the monoclonal antibody SM-3 is selectively exposed in a range of primary carcinomas. Int J Cancer. 1989;43(6):1072–1076.

14. Ho SB, Niehans GA, Lyftogt C, et al. Heterogeneity of mucin gene expression in normal and neoplastic tissues. Cancer Res. 1993;53:641–651.

15. Zhang YM, Liu N, Zhu ZH, Rusckowski M, Hnatowich DJ. Influence of different chelators (HYNIC, MAG3 and DTPA) on tumor cell accumulation and mouse

biodistribution of technetium-99m labeled antisense DNA. Eur J Nucl Med. 2000;27(11):1700–1707.

16. Taylor-PapadimitriouJ, Burchell J, Miles DW, Dalziel M. MUC1 and cancer. Biochim. Biophys. Acta. 1999;1455(2–3):301–313.

17. Vlad AM, Diaconu I, Gantt KR. MUC1 in endometriosis and ovarian cancer. Immunol Res. 2006;36(1–3):229–236.

18. Ferreira CSM, Matthews C, Missailidis S. DNA aptamers that bind to Muc1 tumour marker: design and characterization of Muc1-binding single stranded DNA aptamers. Tumour Biol. 2006;27(6):289–301.

19. Ferreira CSM, Papamichael K, Guilbault G, Schwarzacher T, Gariepy J, Missailidis S. DNA aptamers against Muc1: design of aptamer-antibody sandwich ELISA for early tumour diagnosis. Anal Bioanal Chem. 2008;390(4):1618–2642.

20. Chopra A, Shukla R, Sharma TK. Aptamers as an emerging player in biology. Aptamers Syn Antibodies. 2014;1(1):1–11.

21. Borbas KE, Ferreira CS, Perkins A, Bruce JI, Missailidis S. Design and synthesis of mono- and multimeric targeted radiopharmaceuticals based on novel cyclen ligands coupled to anti-MUC1 aptamers for the diagnostic imaging and targeted radiotherapy of cancer. Bioconjug Chem.2007;18(4):1205–1212.

22. Pieve CD, Perkins AC, Missailidis S. Anti-Muc1 aptamers: radiolabelling with (99m)Tc and biodistribution in Mcf-7 tumour-bearing mice. Nucl Med Biol. 2009;36(6):703–710.

23. Da Pieve C, Blackshaw E, Missailidis S, Perkins AC. PEGylation and biodistribution of an anti-MUC1 aptamer in MCF-7 tumor-bearing mice. Bioconjug Chem. 2012;23(7):1377–1381.

24. Ferreira CS, Cheung MC, Missailidis S, Bisland S, Gariépy J. Phototoxic aptamers selectively enter and kill epithelial cancer cells. Nucleic Acids Res. 2009;37(3):866–876.

25. Hu Y, Duan J, Zhan Q, Wang F, Lu X, Yang XD. Novel MUC1 aptamer selectively delivers cytotoxic agent to cancer cells in vitro. PLoS One. 2012;7(2):e31970.

26. Sá LT, Simmons S, Missailidis S, da Silva MI, Santos-Oliveira R. Aptamer-based nanoparticles for cancer targeting. J Drug Target. 2013;21(5):427–434.

27. Yu C, Hu Y, Duan J, et al. Novel aptamer-nanoparticle bioconjugates enhances delivery of anticancer drug to MUC1-positive cancer cells in vitro. PloS One. 2011;6:e24077.

28. Hamoudeh M, Kamleh MA, Diab R, Fessi H. Radionuclides delivery systems for nuclear imaging and radiotherapy of cancer. Adv Drug Deliv Rev. 2008;60:(12)1329–1346.

29. RobilottaCecil C. A tomografia por emissçao de pósitrons: uma nova modalidade na medicina nuclear brasileira. Ver Panam Salud Publica [Positron emission tomography: a new modality in Brazilian nuclear medicine]. V 20, n. 2/3; 2006. Available from: http://journal.paho.org/uploads/1162234592.pdf. Acesso em 05 de março de 2016. Portuguese.

30. Tonin RS. Cintilografia e PET/CT nas neoplasias malignas [Scintigraphy and PET/CT in malignant neoplasms]. Porto Alegre, Rio Grande do Sul: UFRS; 2011. Portuguese.

31. Santos-Oliveira R. Nanoradiopharmaceuticals: is that the future for nuclear medicine? Curr Radiopharm. 2011;4(2):140–143.

32. Sarcinelli MA. Desenvolvimento de Nanopartícula de Poli(Ácido Lático) contendo Anticorpo Monoclonal marcada com 99mTc para o Diagnóstico Precoce e Tratamento Eficaz do Câncer de Mama [Tese de D.Sc]. Rio de Janeiro, RJ: IMA/UFRJ; 2014. Portuguese.

33. Albernaz M de S, Ospina CA, Rossi AM, Santos-Oliveira R. Radiolabelled nano hydroxyapatite with 99mTc: perspectives to nano radiopharmaceuticals construction. Artif Cells Nanomed Biotechnol. 2014;42(2):88–91.

34. Pinto SR, Sarcinelle MA, de Souza Albernaz M, et al. In vivo studies: comparing the administration via and the impact on the biodistribution of radiopharmaceuticals. Nucl Med Biol. 2014;41(9):772–774.

35. Zhou J, Satheesan S, Li H, et al. Cell-specific RNA aptamer against human CCR5 specifically targets HIV-1 susceptible cells and inhibits HIV-1 infectivity. Chem Biol. 2015;22(3):379–390.

36. Baek JS, Kim JH, Park JS, Cho CW. Modification of paclitaxel-loaded solid lipid nanoparticles with 2-hydroxypropyl-b-ciclodextrin enhances absorption and reduces nephrotoxicity associated with intravenous injection. Int J Nanomedicine. 2015;10:5397–5405.

37. Xu F, Liu F, Zhao H, An G, Feng G. Prognostic significance of MUC1 in various human epithelial cancer: a meta-analysis. Medicine. 2015;94(50):e2286.

38. Gouyer V, Dubuquoy L, Robbe-Masselot C, et al. Delivery of a mucin domain enriched in cysteine residues strengthens the intestinal mucous barrier. Sci Rep. 2015;15(5):9577.

39. Kim GE, Bae HI, Park HU, et al. Aberrant expression of MUC5AC and MUC6 gastric mucins and sialylTn antigen in intraepithelial neoplasm of the pancreas. Gastroenterology. 2002;123(4):1052–1060.

40. Corfield A, Myerscough N, Longman R, Sylvester P, Arul S, Pignatelli M. Mucins and mucosal protection in the gastrointestinal tract: news prospects for mucins in the phatology of gastrointestinal disease. Gut. 2000;47(4):589–594.

41. Lee MJ, Lee HS, Kim WH, Choi Y, Yang M. Expression of mucins and cytokeratins in primary carcinomas of the digestive system. Mod Pathol. 2003;16(5):403–410.

42. Anselmo AC, Gupta V, Zern BJ, et al. Delivering nanoparticles to lungs while avoiding liver and spleen through adsorption on red blood cells. ACS Nano. 2013;7(12):11129–11137.

43. Blanco E, Shen H, Ferrari M. Nanoparticle rational design implementation for overcoming delivery barriers. Nat Biotechnol. 2015;33:941–951.

2.2 **Breast Cancer: Carcinogenesis, Diagnosing And Treatment (Artigo científico publicado)**

Marta de Souza Albernaz, **Fagner Santos do Carmo**, Edward Helal-Neto, Sofia dos Santos Nascimento, Ralph Santos-Oliveira. *Breast Cancer: Carcinogenesis, Diagnosing And Treatment* 2017. 53-64 f. Clinical case report

Summary. Breast cancer is the most common and deadly type of cancer that affects women worldwide. A good anamnesis, allied with a highly accurate diagnosis and a correct interpretation of the data acquired relies the best chance for the patient to receive the best treatment and, in this direction, improve the chance of cure and/or better prognosis. In all the cases, a good theoretical basis is fundamental. Thus, this update review intend to be a primary source on Breast Cancer, paving and consolidating knowledge in the field of breast cancer and helping physicians in their daily difficult task to deal with this disease.

Key words: oncology, education, cancer, female, disease

INTRODUCTION

Cancer

Cancer is generally defined by an uncontrolled, usually rapid cellular proliferation, and therefore does not respond to the common mechanisms of cell cycle control. It is a highly complex, heterogeneous, and multifactorial disease. In several types of tumors, some malignant cells migrate to new sites (metastasis) forming secondary tumors that generally have a large impact on patient's survival. This process of invasion and metastasis begins by a local invasion, extravasation of tumor cells into blood or lymphatic vessels, dissemination, intravasion to distant organs, formation of small tumor cells nodules (micrometastasis) and, growth of macroscopic tumors.

Although there are numerous barriers to the development of cancer, a tumor cell can acquire characteristics that allow it to grow and spread such as sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality,

inducing angiogenesis, and activating invasion and metastasis. Moreover, tumors are surrounded by a repertoire of “normal cells” that contributes to the acquisition of these characteristics, creating a tumor microenvironment. Therefore, tumor growths rely on the intrinsic contribution of intracellular signaling pathways and the complex interaction between components of the tumor microenvironment (1, 2).

Tumors can be divided into benign and malignant according to their biological behavior. Benign tumors do not invade adjacent tissues and grow locally resembling their original tissue. They are rarely life threatening. Malignant tumors, on the other hand, are those rapidly dividing that invade neighboring structures and give rise to metastasis (3).

Breast cancer is the most common cancer among women worldwide. A number of recognized risk factors contribute to the development of breast cancer, including hormone reproduction, age, obesity, alcohol, radiation, benign breast disease and lack of exercise. According to Lv et al (2016), more than 240,000 women developed breast cancer and ~ 40,000 died of the disease in the United States in 2016. Overall, about 1.7 million women were diagnosed in 2012, emphasizing the urgent need for effective and safe therapeutic approaches. Although most breast cancers are slow-growing or indolent, a subgroup acquires an aggressive phenotype for a variety of reasons. Molecular, genotypic and phenotypic studies clearly demonstrate the heterogeneity of breast cancer with multiple subtypes and classifications (3, 4).

Carcinogenesis

Carcinogenesis is a multistep process characterized by genetic alterations that affect key cellular pathways involved in growth and development (5, 6). Oncogenes refer to genes whose alteration cause gainof-function effects. Activated oncogenes, for example, can cause cells designated for apoptosis to survive and proliferate instead. Most oncogenes began as protooncogenes, normal genes involved in cell growth and proliferation or inhibition of apoptosis. When subjected to a genetic mutation, proto-oncogenes are upregulated and can predispose cells to become cancerous. These genes are thus termed oncogenes (7, 8). On the other hand, tumor suppressor genes cause loss-offunction effects that contribute to a malignant phenotype. For example, a tumor suppressor gene can protect a cell from one step to the path to cancer. When this gene is altered it causes a loss or a reduction in its function, allowing the cell to progress to cancer. The effects of these alterations are very complex

because of the high number of changes needed for a cell to become cancerous and the interaction of the biological pathways involved.

Carcinogenesis can be conceptually divided into four stages: tumor initiation, tumor promotion, malignant conversion and tumor progression. The activation of oncogenes and inactivation of tumor suppressor genes are mutational events that result from permanent DNA damage caused for example, by chemical exposures. The accumulation of mutations, and not necessarily the order in which they occur, constitutes multistage carcinogenesis (5, 9).

Tumor initiation is the first stage in which the initial modifications are irreversible genetic damage caused by carcinogens. A chemical carcinogen causes a genetic error, modifying the molecular structure of deoxyribonucleic acid (DNA) that can lead to a mutation during DNA synthesis. These irreversible changes can lead to the activation of oncogenes or inactivation of tumor suppressor genes (7, 10).

Tumor promotion involves the selective clonal expansion of mutated cells. These cells are non-mutagenic, requiring a metabolic activator (oncopromoters) to mediate their biological effects (7, 11).

Malignant conversion is the transformation of the pre-neoplastic cell into one expressing malignant phenotype. The promotion of the tumor contributes to the process of carcinogenesis by the expansion of a population of initiated cells that converge to malignancy. The conversion of a fraction of these cells to malignancy will be accelerated in proportion to the rate of cell division and the amount of dividing cells in the benign tumor or pre-neoplastic lesion. Some components of the diet and prolonged and excessive exposure to hormones are examples of factors that promote the transformation of cells started into malignant ones. The p53 gene located on chromosome 17p13.1 is the most common target of genetic changes in human tumors, being altered in just over 50% of the cases. The homozygous loss of this gene is notable because it can occur in virtually all types of cancer, partly explained by its functional activities, which involve cell cycle arrest and the onset of apoptosis in response to DNA damage (9, 10, 12).

Tumor progression comprises expression of the malignant phenotype and the tendency of malignant cells to acquire more aggressive characteristics over time. In addition, metastasis may involve the ability of tumor cells to secrete proteases that allow invasion beyond the site of the immediate primary tumor (13). At this stage, the cancer is already established, evolving the appearance of the first clinical manifestations of the disease (1, 6, 11, 14).

In all this process, other genetic changes may occur, including activation of oncogenes and functional loss of tumor suppressor genes. Proto-oncogenes can be activated by two

major mechanisms: for example, in the RAS gene family, point mutations are found in highly specific regions of the gene and the MYC, RAF, HER2 and JUN genes may be overexpressed, sometimes involving amplification of chromosomal segments containing these genes. The loss of tumor suppressor gene function normally occurs in a bimodal fashion, and more often involves point mutations in one allele and loss of the second allele by a deletion, recombination event, or non-chromosomal disjunction (1, 15, 16).

The uncontrolled proliferation of cancer cells may lead to the formation of, new blood vessels (angiogenesis), required to the adequate supply of oxygen and nutrients to proliferating tumor cells. The formation of such new blood vessels is important not only in supporting tumor growth, but also provide an opportunity to cancer cells to invade neighboring tissues, enter the circulatory system and begin the metastatic process. When present in the lymphatic and blood vessels, tumor cells can then reach distant organs and proliferate in a secondary place completing the metastasis process (6, 16-18).

Cancer initiation and progression is considered as a multistep process which lately drives malignant transformation of normal cells. However, nowadays, several evidences have suggested that cancer stem cells (CSCs) contribute to the metastatic dissemination of solid tumors. These cells, cancer stem cells (CSCs), are a small cell subpopulation with embryonic characteristics such as self-renewal, high proliferation rate, and the ability to generate heterogenic lineages of cancer cells, are key contributors to the development and progression of the disease (19).

Different theories have been proposed about the origin of CSCs and several hypotheses have been described. One of them states that CSCs arise from stem cells. In fact, stem cells can divide to produce copies of themselves, or self-renew and, are pluripotent (able to differentiate into most mature cell types). Therefore, an unsuitable mutation may lead to transformation of dormant normal stem cells to cancer stem cells (CSCs) (20, 21). According to another, CSCs may arise from progenitor cells. Indeed, the differentiation pathway from a stem cell to a differentiated cell usually involves intermediate cells types, called progenitor cells that are more abundant in adult tissue than are stem cells. Progenitor cells usually divide to produce mature cells and retain a partial capacity for selfrenewal. Thus, this property has led to the theory that mutations in progenitor cells could lead to a source of CSCs (22, 23).

Some researchers have suggested that CSCs could arise from mature, fully differentiated cells. In this theory, a adult somatic cell could undergo several mutations and de-differentiate to become in a more stem-like state. The genetic mutations would need to

drive not only the de-differentiation process, but also the self-renewal of proliferating cells (24, 25).

Because most deaths from cancer patients are from metastasis, a better understanding of the mechanisms of tumor metastasis is important for developing more effective therapeutic strategies.

Breast cancer

Breast cancer is the most common cancer among women worldwide (both in developing and developed countries). In 2012, about 1.67 million new cases breast cancer were detected worldwide, accounting for approximately 25% of all cancers diagnosed in women. Still in 2012, 522,000 deaths from breast cancer were recorded in women worldwide. These deaths account for 15% of all cancer deaths in women. Breast cancer is the second leading cause of cancer death in developed countries (198,000 deaths) behind only lung cancer (26, 27). Women with breast cancers can be successfully treated if diagnosed in an early stage of the disease.

Histological classification of breast tumors

Breast cancers usually are epithelial tumors of ductal or lobular origin and are classified as follows: Ductal carcinoma in situ (DCIS), Lobular carcinoma in situ, Invasive ductal carcinoma (ductal breast cancer), Invasive lobular carcinoma, Medullary carcinoma, Mucinous (colloid) carcinoma, Tubular carcinoma, Papillary carcinoma, Metaplastic breast cancer (MBC), Phyllodes tumors, Mammary Paget disease (MPD), Inflammatory breast cancer.

Invasive ductal carcinoma

Most breast tumors originate in the ductal epithelium (about 80%) and are known as invasive ductal carcinoma. The “invasive ductal carcinoma” refers to cancer that has broken through the wall of the milk duct and begun to invade the adipose tissue of the breast. Over

time, invasive ductal carcinoma can spread to the lymph nodes and possibly to other areas of the body. The diagnosis of invasive ductal carcinoma is made by the exclusion of recognized specific breast cancer. When the lesion does not fulfill the diagnostic criteria for any other special types of mammary carcinoma, tumor has been classified as invasive ductal carcinoma without other specification. The tumor is formed by the proliferation of epithelial elements with relatively high cytological atypia, which is characterized by the presence of many epithelial cells in the cytoplasm with a variable tendency to form pseudo-glandular or ductlike structures, and with variable mitotic activity (28, 29). The cytological characteristics vary widely and can be found from small cells with homogeneous nuclei to large cells with irregular and hyperchromatic nuclei. On the margins of the tumor mass, neoplastic cells infiltrate into the stroma and fibro-adiposal tissue, and there is often an invasion of the perivascular and perineural spaces, as well as of the blood and lymphatic vessels (30, 31).

Lobular carcinoma

Lobular carcinoma, often called, invasive lobular carcinoma, is the second most common type of breast cancer after invasive ductal carcinoma. It occurs in the breast lobules of the mammary gland, and can broke through the wall of the lobes and invade the tissue of the breast (10% of the cases).This type of cancer has a good prognosis, with a 10-years overall survival in 80-90% of the women. It is characterized by a risk of bilaterality and high rate of late systemic recurrence. They are often distinguished by their molecular physiology, since they often have E-cadherin loss and are typically positive for estrogen and progesterone receptors. In addition, several distinctive genomic alterations were observed in lobular tumors, including 1q gain and chromosome 16q20. A large study of lobular characteristics also categorized several mutations in the PTEN, TBX3 and FOXA1 genes that typify lobular carcinomas (31, 32).

Tubular carcinomas

Tubular adenomas are rare benign neoplasms, representing 0.13-1.7% of benign breast lesions. Tubular adenomas are circumscribed, unencapsulated, slowgrowing, firm, movable, and small to medium-sized female breast lesions consisting of densely packed regular round

tubules. Young women of reproductive age (15-49 years) are commonly affected. The upper and outer quadrant of the breast is the most preferred site. Recurrence or increased risk of cancer is not reported from cases of tubular adenoma (33).

Mucinosis carcinoma

This type of cancer represents 1-4% of all cases of breast cancers. It usually manifests in postmenopausal women, has a good prognosis, with a 10-year survival rate in 80% to 90% of cases, often associated with mutations in the BRCA1 gene (34).

Marrow carcinoma

This type represents less than 5% of all invasive breast cancers. It is more common in young women and is associated with abnormalities in the BRCA1 gene. It has a better prognosis than ductal carcinomas (12).

Micropapillary carcinoma

It is a distinct form of mammary carcinoma characterized by the proliferation of malignant cells in micropapillary arrays within cystic spaces in the breast stroma, without epithelial or endothelial lining, with frequent metastasis in the diagnostic phase (35). The incidence of IMPC ranges from 3 to 6 % of all primary breast cancers. It is an important subtype due to its unique features such as high proclivity to lymphovascular invasion, lymph node metastasis, local recurrence, and distant metastasis, thus exhibiting a more aggressive behavior with a poorer prognosis than invasive ductal carcinoma (36, 37).

Carcinoma papillaryis

With an incidence ranging from 1.1% to 1.7% of all malignant tumors of the breast, is considered a rare type. In most cases, these types of tumors are diagnosed in older women who have already been through menopause. Histopathological features include low grade

cellular atypia, intracellular or extracellular mucin deposition, and solid papillary growth pattern, as well as neuroendocrine differentiation (38, 39).

Metaplastic carcinoma

Due to the great heterogeneity and the different evolutionary profile, this group was sub classified. The fibromatosis-simile subtype presents a differential diagnosis with lesions and benign fusocellular tumors, especially in needle biopsies with limited samples or when they occur associated with sclerosing lesions, radial scars or papillomas. Immunohistochemically study for cytokeratins (CK), especially those of high molecular weight (34β E-12, CK-5 or CK-5/6), and p63 aids in the differential diagnosis and positively affects tumor spindle cells. Differential diagnoses include benign fusocellular lesions (fibromatosis, nodular fasciitis, myofibroblastoma and needle biopsies after needle biopsy) and low-grade fusocellular sarcomas (40, 41).

The spread of mammary carcinomas is by local invasion (skin, nipple, muscle or chest wall), lymphatic or hematogenous. In 30% to 50% of the cases there is axillary lymph node involvement at the time of diagnosis, and regional metastases indicate distant and systemic metastatic potential. Women with 1-3 compromised lymph nodes have 60% survival at 10 years; this rate reduces to 20% in women who had 3-4 or more lymph nodes with metastases at the time of diagnosis. Systemic metastases generally occur in the lungs, bones, liver, adrenal glands, ovaries, and the central nervous system. About 30% of women without axillary metastases develop systemic metastasis later, indicating that a large proportion of breast carcinomas are already systemic diseases at the time of diagnosis (32, 42).

Molecular classification of breast tumors

There are 6 intrinsic subtypes according to their gene expression pattern: luminal A, luminal B, HER-2, basal-like, normal-like, and claudin-low overexpression tumors.

Luminal

Approximately 75% of breast cancers are positive for Estrogen receptor (ER) and/or Progesterone receptor (PR). This type of tumor encodes typical proteins of luminal epithelial cells so they are termed the luminal group. The luminal tumor cells look the most like cells of breast cancers that start in the inner (luminal) cells lining the mammary ducts. Two main luminal-like subclasses corresponding to Luminal A and Luminal B have been described so far (3, 8, 43).

Luminal A

About 30-70 percent of breast cancers are luminal A tumors. These tumors frequently have low histological grade, low degree of nuclear pleomorphism, low mitotic activity and include special histological types (i.e., tubular, invasive cribriform, mucinous and lobular) with good prognosis. They areoriginatedin epithelial cells differentiated from ducto-lobular lumens, presenting overexpression of estrogen receptor (ER) and progesterone (PR), and genes that are activated by hormonal binding, such as the BCL2 gene, which regulates apoptosis, and the GATA-3 transcription factor, and absence of HER2. The Ki67 evaluation

shows a low proliferation rate (<14%). Because luminal A tumors tend to be ER-positive, treatment for these tumors often includes hormone therapy. Patients with luminal-A breast cancer have a good prognosis and the relapse rate is significantly lower than the other subtypes (3, 43).

Luminal B

Luminal-B tumors comprise 15%-20% of breast cancers and have a more aggressive phenotype in comparison to Luminal A. They present higher histological grade, proliferative index and a worse prognosis. Luminal B tumors have a higher recurrence rate and lower survival rates after relapse compared to luminal-A subtype. Luminal B tumors tend to be ER-positive. They may be HER2-negative or HER2-positive. Approximately 30% of HER2-positive tumors defined by immunohistochemistry are assigned to the luminal-B subtype. This

tumor is also sensitive to hormone therapy, although to a lesser extent, and Trastuzumab (TZB) can be used successfully if it is HER2 positive (3, 43).

HER2

The human epidermal growth factor receptor-2 is a member of the family of four membrane tyrosine kinases. The HER2 receptor is encoded by the HER2 gene, which is a proto-oncogene mapped in chromosome 17q21. HER-2 is amplified in 15-20% of breast carcinomas. Its overexpression is associated with a more aggressive tumor phenotype, but more responsive to monoclonal targeted therapy (Herceptin). HER2 positivity confers a more aggressive biological and clinical behavior. Morphologically, these tumors are highly proliferative, 75% have a high histological and nuclear grade and more than 40% have p53 mutations. Nearly half of HER2-positive breast cancers are positive for ER but they generally express lower ER levels (44-46).

Basal-Like

The basal-like subtype is highly aggressive and, therefore, of particular clinical relevance (3, 43). Basallike breast cancers are more likely to occur in younger women, and are associated with mutations in the breast cancer susceptibility gene (BRCA1). They are characterized by high tumor rate, proliferation rate, frequency of recurrence and the presence of p53 mutations. Morphologically, it is characterized by a high histological grade, by a high mitotic index, by the presence of central necrotic areas and by the prominent lymphocytic infiltrate (8). It is estimated that 15 to 20% of breast carcinomas are basal-like. They are undifferentiated or undifferentiated lesions with high proliferation rates. For the most part (70-80%), they are triple-negative tumors by immunohistochemically reaction, with negativity of ER, PR and HER2.

It is important to notice that despite the similarity, basal-like and triple-negative breast cancer terms are not synonymous: the first one is defined by gene expression in DNA microarrays, and the second one, by immunohistochemically criteria. The panel of markers proposed for the classification of the basallike type would be the absence of expression of RE, PR and HER2, expression of high molecular weight/basal cytokeratin's, CK5/6, 14 or 17, and

expression of EGFR (HER1). Triple negative breast cancer with basal-like features lack expression of the biomarkers ER, PR, and HER2, but commonly express high molecular-weight 'basal' cytokeratin (CK5⁄6, CK14, and CK17) epidermal growth factor receptor (EGFR), vimentin, p-cadherin, αB-crystallin, fascin, and caveolins 1 and 2 (47).

Basal-like tumors, despite being more aggressive, are more responsive to neoadjuvant chemotherapy (8, 48). BRCA1 dysfunction, seems to represent a mechanism that generates basal-like and triple-negative tumors; Thus, it may be inferred that at least a part of these are incompetent in the DNA repair mechanism involved in the homologous recombination pathway; this makes these cells more dependent on repair pathways by the enzymes of poly ADP-ribose polymerase (PARP) (3, 43).

Normal-like

The existence of the normal-like subtype is controversial. The term was used because the genes expressed therein are usually shared with normal epithelial tissue. However, it is not clear whether this subtype even exists or whether its determination was simply due to contamination with normal tissue samples (49, 50).

Claudin-low

Recognized in 2007, they are also triple-negative tumors, with low expression of claudin genes 3, 4 and 7, and loss of E-cadherin. Its frequency is estimated to be 5% of all breast carcinomas and its origin is linked to cells very close to the primitive stem-mammary cells (49, 51). Claudins are transmembrane proteins involved in adhesion between cells, and the regulation of some of them is associated with breast cancer, apparently by epigenetic silencing, facilitating cell migration and tissue invasion (50, 51).

In claudin-low carcinomas there are no markers of luminal differentiation; on the contrary, these forms are rich in markers of stem cells, cancer initiating cells, epithelial-mesenchymal transition, and genes associated with the immune response. It is the tumor whose cells most resemble stem cells (49, 52). They have a high histological grade, little differentiation and show a marked lymphocytic infiltration (52).

Risk factors

Breast cancer is a type of cancer considered multifactorial, involving biological-endocrine factors, reproductive life, behavior and lifestyle, aging, factors related to women's reproductive life, family history, high density of breast tissue (ratio between glandular tissue and adipose tissue of the breast) are the most wellknown risk factors for the development of breast cancer. In addition, alcohol consumption, excess weight (due to IGF-1 genes, such as IGF-1, as well as changes in serum levels of hormones such as insulin and leptin), sedentary lifestyle, and exposure to ionizing radiation are also considered as potential agents for the development of this cancer (53).

However, breast cancer observed in young women has very different clinical and epidemiological characteristics than those seen in older women. They are generally more aggressive, have a high rate of BRCA1 and BRCA2 gene mutations, and overexpress the human epidermal growth factor receptor 2 (HER2) genes (54-56).

Changes in genes, such as the BRCA family, increase the risk of developing breast cancer (57, 58). Factors related to women's reproductive life are also linked to the risk of developing this type of neoplasia. Early menarche (age at first menstruation less than 12 years), late menopause (after age 55), nulliparity, and having the first child after the age of 30 contribute to an increased risk of breast cancer. On the other hand, breastfeeding is associated with a lower risk of developing this type of cancer (57).

The practice of physical activity and healthy eating with maintenance of body weight are associated with an approximately 30% reduction in the risk of developing breast cancer. Postmenopausal obesity is also considered a risk factor, but this risk decreases with the practice of regular physical activity (59-61).

Early detection aims to identify cancer in the early stages, in which the disease may have a better prognosis. Is important to notice that early detection of breast cancer do not reduce incidence but may reduce the mortality (62, 63). To solve this problem, different non-invasive imaging technologies are researched for both early diagnosis and to monitor the onset of metastasis. These techniques include, Positron Emission Tomography (PET) or Single-Photon Emission Computed Tomography (SPECT), Magnetic Resonance Imaging (MRI), Mammography, Ultrasonography (US), Computerized Tomography (CT), and Optical Imaging (bioluminescence and/or fluorescence imaging) (62, 64-67).

Mammography

Mammography is considered the standard method of early detection of breast cancer and diagnosis, but it has limitations, such as low sensitivity in dense breasts. Breast cancer is a heterogeneous disease, with variation of biological behavior, different growth rates and different metastatic potential (62). Slow-growing tumors are more easily detected in the tracing, but there may be no benefit in their early detection. In more aggressive cancers, early detection with mammography, in addition to being more difficult, may not be effective due the rapid growth rate and the potential to generate metastases in a short time, even when the primary tumors are still small (64-66, 68).

Ultrasonography

Ultrasonography is, alongside mammography, the most important imaging method in the diagnostic investigation of suspected mammary alterations, and the two methods are seen as complementary in the approach of different clinical situations. Ultrasonography is used to detect, characterize and guide the biopsy of breast lesions. It presents two important advantages on mammography: the absence of the use of ionizing radiation and the fact that its diagnostic acuity does not depend on the mammary density (66. 69). The US has known limitations that compromise its potential as a screening method for breast cancer. Among these limitations, there is the dependence on the presence and experience of the attending physician, the greater difficulty in standardizing examination techniques and interpretation criteria, and the difficulty in detecting micro-calcifications (70).

Magnetic Resonance

Magnetic resonance imaging is effective for the screening of dense breasts and identification of addi tional occult lesions in the ipsilateral or contralateral region of the breast. It may also help to determine if lumpectomy or mastectomy (unilateral or bilateral) is the best treatment. Although MRI is highly sensitive (94% to 100%), specificity is low (37% to 97%). It is suggest that the combination of MRI and mammography screening could

improve the chances of early detection of breast cancer. However, magnetic resonance imaging is not routinely used in screening due the price of the exam (63, 66, 71).

Nuclear Medicine

Nuclear Medicine has been used in the last 40 years as in diagnostic imaging, decision-making regarding the treatment or monitoring the response to treatment. Imaging radiopharmaceutical could evaluate organ physiology, distinguishing between normal and neoplastic tissue (62, 66).

The most commonly used radiopharmaceutical breast imaging is 99mTc-sestamibi. This radiopharmaceutical enters the cell by passive diffusion of the extracellular compartment into the cytoplasm and accumulates into the mitochondria, considering that most of the malignant cells have a higher mitochondrial intracellular, it accumulation indicates the tumor presence (66, 72-75). Studies on the sensitivity and specificity of MIBI for detection of breast cancer demonstrated a sensitivity of 96% in detection, but showed a moderate specificity (59%) (66, 76).

Treatment

Surgery

Surgery is a common treatment for breast cancer, and its main purpose is to remove as much of the cancer as possible. There are two main types of surgery to remove breast cancer. In the breast-conserving surgery (also called a lumpectomy, quadrantectomy, partial mastectomy, or segmental mastectomy) only the part of the breast containing the cancer is removed. The aim of this type of surgery is to remove the cancer as well as some surrounding normal tissue. How much of the breast is removed depends on the size and location of the tumor and other factors. The mastectomy on the other hand, is a kind of surgery where the entire breast is removed, including all of the breast tissue and sometimes other nearby tissues. There are several different types of mastectomies. Some women may also get a double mastectomy, in which both breasts are removed (77, 78).

Radiotherapy

Radiation after BCS (Breast Conserving Surgery) for early as well as locally advanced tumor after neoadjuvant chemotherapy (NACT) is now considered as an integral part of BCT (Breast Conserving Therapy) whereas post mastectomy radiation (PMRT) to chest wall and or regional area is considered beneficial for a select group of high risk patients (79).

Radiation therapy is a treatment with high-energy rays (such as x-rays) or particles that will kill tumor cells. Two main types of radiation therapy are conventionally used to treat breast cancer: the external beam radiation (a type of radiation coming from a machine outside the body), and brachytherapy (a radioactive source put inside the body). The external beam radiation is the most common type of radiation therapy to treat breast cancer. The radiation beam is usually generated by a linear accelerator capable of producing high-energy X-rays and electrons. Different types of external beam therapy are used for specific types of cancer. For example, Three-Dimensional Conformal Radiation Therapy (3D-CRT) is used when tumors are not regular (different shapes and sizes) and uses special imaging techniques to show the size, shape and location of the tumor. This technique precisely tailors the radiation beams to the size and shape of the tumor allowing nearby normal tissue to receive less radiation. The Intensity Modulated Radiation Therapy (IMRT) is a specialized form of 3D-CRT in which the beam can be broken up into many “beamlets” and the intensity of each beamlet can be adjusted individually. This allows the radiation to be more exactly shaped to fit the tumor and limits the amount of radiation that is received by healthy tissue near the tumor. The Proton Beam Therapy uses protons rather than X-rays to treat cancer and more effectively reduces the radiation dose to nearby healthy tissue. In the Neutron Beam Therapy, a neutron beam is often used to treat cancers radioresistants to the conventional X-ray radia tion therapy. The Image Guided Radiation Therapy uses imaging techniques (CT, ultrasound or X-rays) to increase the delivery of radiation to the tumor site in cases were tumors can move between treatments because of differences in organ filling or movements while breathing. Still, which areas need radiation depends on whether mastectomy or breast-conserving surgery (BCS) was done and whether the cancer has reached nearby lymph nodes (81, 82).

Although radiation provides significant benefit to many women with breast cancer, it is also associated with risks of toxicity, including cardiac and pulmonary toxicity, lymphedema, and secondary malignancy.

Chemotherapy

Chemotherapy is a type of cancer treatment that uses one or more anti-cancer drugs (chemotherapeutic agents) as part of a standardized chemotherapy regimen. Chemotherapy may be given before surgery, after surgery or for the main treatment of advanced breast cancers. The main purpose of the neoadjuvant therapy, also referred to as preoperative or primary chemotherapy, is to reduce the size of the primary tumor, eventually allowing radical or more conservative surgical interventions. The adjuvant chemotherapy (after surgery) on the other hand is used after surgery to try to kill any cancer cells that may have been left behind or spread but can't be seen, even on imaging tests. Chemotherapy can also be used as the main treatment for metastatic breast cancer and cannot be surgically removed (83-86).

The most commonly used drugs for adjuvant and neoadjuvant chemotherapy include: Anthracyclines such as doxorubicin (Adriamycin) and epirubicin (Ellence); Taxanes, such as paclitaxel (Taxol) and docetaxel (Taxotere); 5-fluorouracil (5-FU); Cyclophosphamide (Cytoxan) and; Carboplatin (Paraplatin).

Most often combinations of two or three of these drugs are used. For advanced breast cancer on the other hand, a single combination is usually utilized and chemotherapeutic drugs include: Docetaxel, Paclitaxel, Platinum agents (cisplatin, carboplatin), Vinorelbine (Navelbine), Capecitabine (Xeloda), Liposomal doxorubicin (Doxil), Gemcitabine (Gemzar), Mitoxantrone (Novantrone), Ixabepilone (Ixempra), Albumin-bound paclitaxel (nab-paclitaxel or Abraxane) or Eribulin (Halaven) (83-86).

Hormone therapy

Hormone therapy, such as anti-estrogen therapy and estrogen ablation, is the treatment of choice for patients with breast cancer expressing estrogen receptors (ER) and/or progesterone receptors (PR). The clinical usefulness of hormone therapy has been proven in the prevention and used after surgery (as adjuvant therapy) and sometimes before surgery (as neoadjuvant therapy) as well (87).

The ER and PR were the first predictive biomarkers recommended for routine clinical use in breast cancer. They are used to distinguish patients who have little or no chance of benefiting from hormone therapy from those who do have some reasonable chance. Once a tumor has been defined as having ER and/or PR expression, a number of potential strategies

to target the hormonal pathway can be used. For example, tamoxifen acts as an antagonist of the ER (by interrupting the transcription of estrogen-regulated genes) and disrupts the proliferative effects of estrogen in the breast. The fulvestrant, similarly acts at the level of the estrogen receptor, but in contrast to tamoxifen only has antagonist activities because it leads to the degradation of the ER protein with loss of ER and subsequent PR expression. Several strategies to produce estrogen deprivation are also used to treat breast cancer such as suppression of ovarian estrogen production in premenopausal women or the use of aromatase inhibitors in postmenopausal women. Moreover, high dose steroids (including estrogen or progesterone) can paradoxically also has an antibreast cancer effect. Therefore, the selection of hormonal therapy is typically based on several factors including menopausal status and side effect profile (88, 89).

Targeted therapies

Clinical trials investigating new drugs and therapeutic combinations have led to promising advances in breast cancer therapy.

The epidermal growth factor receptor 2 (ErbB2, HER2), a member of the growth factor receptor family (HER1/2/3/4), has been one of the most success ful targets discovered in breast cancer. HER2-targeted therapy using the humanized monoclonal antibody trastuzumab has significantly improved disease-free and overall survival in early stage HER2-positive breast cancer. Nowadays, trastuzumab is considered a first-line treatment for advanced HER2-positive breast cancers (90).

In addition to hormone and HER2-targeted therapies recent preclinical studies have shown several targetable pathways that overcome resistance and are currently being used in the clinical setting. The mTOR inhibitor everolimus and the CDK4/6 inhibitor palbociclib have been approved in HER2-positive metastatic breast cancer and improved disease-free survival. The combination of pertuzumab with Trastuzumab and taxanes further improved disease free survival in HER2-positive breast cancer. However, patient selection and predictive biomarker development remains a big challenge for targeted therapy development in breast cancer (91-93).

References

1. Tong HV, Brindley PJ, Meyer CG, et al. Parasite Infection, Carcinogenesis and Human Malignancy. EBioMedicine 2017; 15: 12-23.

2. Yu K, Sang QXA, Lung PY, et al. Personalized chemotherapy selection for breast cancer using gene expression profiles. Scientific Reports 2017; 7(43294); 10.

3. Narayanan R, Dalton JT. Androgen Receptor: A Complex Therapeutic Target for Breast Cancer. Cancers 2016; 8(108):17.

4. Lv Q, Meng Z, Yu Y, et al. Molecular Mechanisms and Translational Therapies for Human Epidermal Receptor 2 Positive Breast Cancer. International Journal of Molecular Sciences 2016; 17(12).

5. Delpu Y, Cordelier P, Cho WC, et al. DNA Methylation and Cancer Diagnosis. International Journal of Molecular Sciences 2013: 14(7): 15029-58.

6. Qian CN, Tan MH, Yang JP, et al. Revisiting tumor angiogenesis: vessel co-option, vessel remodeling, and cancer cellderived vasculature formation. Chinese Journal of Cancer 2016; 35(10): 6.

7. Barcellos-Hoff MH, Lyden D, Wang TC. The evolution of the cancer niche during multistage carcinogenesis. Nature Reviews Cancer 2013; 13(7): 511-8.

8. Lo P, Wolfson B, Zhou Q. Cancer stem cells and early stage basal-like breast cancer. World Journal of Obstetrics and Gynecology 2016; 5(2): 150-61.

9. Willis RE. Targeted Cancer Therapy: Vital Oncogenes and a New Molecular Genetic Paradigm for Cancer Initiation Progression and Treatment. International Journal of Molecular Sciences 2016; 17(9): 23.

10. Feller L, Khammissa RAG, Lemmer J. Biomechanical cell regulatory networks as complex adaptive systems in relation to cancer. Cancer Cell International 2017; 17(16): 6.

11. Mertz TM, Harcy V, Roberts SA. Risks at the DNA Replication Fork: Effects upon Carcinogenesis and Tumor Heterogeneity. Genes (Basel) 2017; 8(46): 21.

12. Wang XX, Jiang YZ, Liu XY, et al. Difference in characteristics and outcomes between medullary breast carcinoma and invasive ductal carcinoma: a population based study from SEER 18 database. Oncotarget 2016; 7(16): 22665-73.

13. Mistry DAH, French PW. Circulating Phospholipids as Biomarkers of Breast Cancer: A Review. Breast Cancer 2016; 10: 191-6.

14. Falco M, Palma G, Rea D, et al. Tumour biomarkers: homeostasis as a novel prognostic indicator. Open Biology 2016; 6(12): 11.

15. Barbosa RCC, Da Costa DM, Ellen D, et al. Interaction of MTHFR C677T and A1298C, and MTR A2756G Gene Polymorphisms in Breast Cancer Risk in a Population in Northeast Brazil. Anticancer Research November 2012; 32(11): 4805-11.

16. Zhou Z, Hick DG. HER2 - Amplification or Overexpression in Upper GI Tract and Breast Cancer with Clinical Diagnosis and Treatment. In Oncogene and Cancer – From Bench to Clinic. Ed. Yahwardiah Siregar, 2013.

17. Weston A, Harris CC. Multistage carcinogenesis. In Holland-Frei Cancer Medicine, 6th edition. B.C. Decker; 2003.

18. Maciejczyk A. New prognostic factors in breast cancer. Advances in Clinical and Experimental Medicine 2013; 22(1): 5-15.

19. Bozorgi A, Khazaei M, Khazaei MR. New Findings on Breast Cancer Stem Cells: A Review. J Breast Cancer 2015; 18(4): 303-12.

20. Hartwig FP, Nedel F, Collares T, et al. Oncogenic somatic events in tissue-specific stem cells: a role in cancer recurrence? Ageing Res Rev 2014; 13: 100-6.

21. Hope KJ, Jin L, Dick JE. Acute myeloid leukemia originates from a hierarchy of leukemic stem cell classes that differ in self-renewal capacity. Nat Immunol 2004; 5(7): 738-43.

22. Yamashita T, Wang XW. Cancer stem cells in the development of liver cancer. J Clin Invest 2013; 123(5): 1911-8.

23. Kucia M, Ratajczak MZ. Stem cells as a two-edged sword-from regeneration to tumor formation. J Physiol Pharmacol 2006; 57(7): 5-16.

24. Yu J, Vodyanik MA, Smuga-Otto K. et al. Induced pluripotent stem cell lines derived from human somatic cells. Science 2007; 318(5858): 1917-20.

25. Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007; 131(5): 861-72.

26. Desantis C, Ma J, Bryan L, et al. Breast cancer statistics, 2013. Cancer J Clin 2014; 64(1): 52-62.

27. Ferlay J, Soerjomataram I, Dikshit R. et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. International Journal of Cancer 2015; 136(5): E359-86.

28. Malhotra GK, Zhao X, Band H, et al. Histological, molecular and functional subtypes of breast cancers. Cancer Biology and Therapy 2010; 10(10): 955-60.

29. Cadoo KA, Mcardle O, O'Shea AM, et al. Management of unusual histological types of breast cancer. Oncologist 2012; 17(9): 1135-45.

30. Sabatier R, Sabiani L, Zemmour C, et al. Invasive ductal breast carcinoma with predominant intraductal component: Clinicopathological features and prognosis. Breast 2016; 27:8-14.

31. Fu D, Zuo Q, Huang Q, et al. Molecular Classification of Lobular Carcinoma of the Breast. Scientific Reports 2017: 7(43265).

32. Viale G. The current state of breast cancer classification. Annals of Oncology 2012; 23 (Suppl 10): 207-10.

33. Sengupta S, Pal S, Biswas BK, et al. Evaluation of Clinico-Radio-Pathological Features of Tubular Adenoma of Breast: a Study of Ten Cases with Histopathological Differential Diagnosis. Iranian Journal of Pathology 2015; 10(1): 17-22.

34. Naqos N, Naim A, Jouhadi H, et al. Mucinous carcinoma of the breast: Clinical, biological and evolutive profile. Cancer Radiotherapie 2016; 20(8): 801-4.

35. Yang YL, Liu BB, Zhang X, et al. Invasive micropapillary carcinoma of the breast: an update. Archives of Pathology and Laboratory Medicine 2016; 140(8): 799-805.

36. Chen L, Fan Y, Lang RG, et al. Breast carcinoma with micropapillary features: clinicopathologic study and long-term follow-up of 100 cases. Int J Surg Pathol 2008; 16: 155-63.

37. Kuroda H, Sakamoto G, Ohnisi K, et al. Clinical and pathologic features of invasive micropapillary carcinoma. Breast Cancer 2004; 11(2): 169-74.

38. Okubo Y, Okubo T, Okubo Y, et al. Neuroendocrine Differentiation in Breast Cancer: Clinicopathological Significance of Bcl-2 Positive Solid Papillary Carcinoma. Case Reports in Medicine 2016; 2016, ID 9501410: 6.

39. Wei S. Papillary Lesions of the Breast: An Update. Arch Pathol Lab Med 2016; 140(7): 628-43. doi: 10.5858/arpa. 2015-0092-RA. Review.

40. Sinn HP, Kreipeb H. A Brief Overview of the WHO Classification of Breast Tumors, 4th Edition, Focusing on Issues and Updates from the 3rd Edition. Breast Care 2013; 8: 149-54.

41. Ydiner A, Sen F, Tambas M, et al. Metaplastic Breast Carcinoma Versus Triple-Negative Breast Cancer. Survival and Response to Treatment. Medicine (Baltimore) 2015; 94(52): e2341.

42. Schwartz TL, Mogal H, Papageorgiou C, et al. Metaplastic breast cancer: histologic characteristics, prognostic factors and systemic treatmentstrategies. Exp Hematol Oncol 2013; 2(1): 31.

43. Tsutsui S, Ohno S, Murakami S, et al. Prognostic significance of the coexpression of p53 protein and c-erbB2 in breast cancer. Am J Surg 2003; 185(2): 165-7.

44. Barnes Cj, Kumar R. Biology of the epidermal growth factor receptor family. Cancer Treat Res 2004; 119: 1-13.

45. Gutierrez C, Schiff R. HER2: biology, detection, and clinical implications. Arch Pathol Lab Med 2011; 135(1): 55-62.

46. Reis-Filho JS, Tutt AN. Triple Negative Tumors: a Critical Review. Histopathology 2008; 52: 108-18.

47. Foulkes WD, Smith I, Reis-Filho JS. Triple-negative breast cancer. New England Journal of Medicine 2010; 363: 1938-48.

48. Prat A, Perou CM. Deconstructing the molecular portraits of breast cancer. Molecular Oncology 2011; 5: 5-23.

49. Rivenbark AG, O'Connor SM, Coleman WB. Molecular and cellular heterogeneity in breast cancer. Challenges for personalized Medicine. American Journal of Pathology 2013; 183: 1113-24.

50. Prat A, Parker JS, Karginava O, et al. Plenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. Breast Cancer Research 2010; 12: R68.

51. Lehman BD, Pietenpol J. Identifications and use of biomarkers in treatment strategies for triple negative breast cancers subtypes. Journal of Pathology 2014; 232: 142-50.

52. Howell A, Anderson AS, Clarke RB, et al. Risk determination and prevention of breast cancer. Breast Cancer Res 2014; 16: 446.

53. Venkitaraman A.R. Functions of BRCA1 and BRCA2 in the biological response to DNA damage. Journal of Cell Science 2001; 114(20): 3591-8.

54. Ren Z, Li Y, Hameed O, et al. Prognostic factors in patients with metastatic breast cancer at the time of diagnosis. Pathology, Research and Practice 2014; 210(5): 301-6.

55. Brenner DR, Brockton NT, Kotsopoulos J, et al. Breast cancer survival among young women: a review of the role of modifiable lifestyle factors. Cancer Causes Control 2016;27: 459-72. 56. Her J, Lee NS, Kim Y, et al. Factors forming the BRCA1-A complex orchestrate BRCA1 recruitment to the sites of DNA damage. Acta Biochimica et Biophysica Sinica 2016; 48(7): 658-64.

57. Mehrgou A, Akouchekian M. The importance of BRCA1 and BRCA2 genes mutations in breast cancer development. The Medical Journal of the Islamic Republic of Iran 2016; 30(369): 12.

58. Bandera EV, Maskarinec G, Romieu I, et al. Racial and Ethnic Disparities in the Impact of Obesity on Breast Cancer Risk and Survival: A Global Perspective. Advances in Nutrition 2015; 6(6): 803-19.

59. Matthews SB, Thompson HJ. The Obesity-Breast Cancer Conundrum: An Analysis of the Issues. International Journal of Molecular Sciences 2016; 17(6): 19.

60. Parsa Y, Mirmalek SA, Kani FE, et al. A Review of the Clinical Implications of Breast Cancer Biology. Electronic Physician 2016; 8(5): 2416-24.

61. De Abreu FB, Wells WA, Tsongalis GJ. The Emerging Role of the Molecular Diagnostics Laboratory in Breast Cancer Personalized Medicine. The American Journal Pathology 2013; 183(4): 1075-83.

62. Wesoła M, Jeleń M. The Diagnostic Efficiency of Fine Needle Aspiration Biopsy in Breast Cancers – Review. Advances in Clinical and Experimental Medicine 2013; 22(6): 887-92.

63. Choi YE, Kwak JW, Park JW. Nanotechnology for early cancer detection. Sensors (Basel) 2010; 10(1): 428-55.

64. Park SH, Kim MJ, Park BY, et al. Impact of Preoperative Ultrasonography and Fine-Needle Aspiration of Axillary Lymph Nodes on Surgical Management of Primary Breast Cancer. Annals of Surgical Oncology March 2011; 18(3):738-744.

65. Vercher-Conejero JL, Pelegrí-Martinez L, Lopez-Aznar D, et al. Positron Emission Tomography in Breast Cancer. Diagnostics 2015; 5(1): 61-83.

66. Mollard S, Fanciullino R, Giacometti S, et al. In Vivo Bioluminescence Tomography for Monitoring Breast Tumor Growth and Metastatic Spreading: Comparative Study and Mathematical Modeling. Scientific Reports 2016; 6: 10.

67. Pettersson A, Graff RE, Ursin G, et al. Mammographic Density Phenotypes and Risk of Breast Cancer: A Metaanalysis. J Natl Cancer Inst 2014; 106(5): dju078. 68. Gold LS, Klein G, Kessler L, et al. The emergence of diagnostic imaging technologies in breast cancer: discovery, regulatory approval, reimbursement, and adoption in clinical guidelines. Cancer Imaging 2012; 12(1): 13-24.

69. Elmore JG, Armstrong K, Lehman CD, et al. Screening for Breast Cancer. The Journal of the American Medical Association 2005; 293(10): 1245-56.

70. Heywang-Köbrunner SH, Schreer I, Heindel W. Imaging Studies for the Early Detection of Breast Cancer. Deutsches Ärzteblatt International 2008; 105(31-32): 541-7.

71. Lei L, Wang X, Chen Z. PET/CT Imaging for Monitoring Recurrence and Evaluating Response to Treatment in Breast Cancer. Advances in Clinical and Experimental Medicine 2016; 25(2): 377-82.

72. Aktolun C, Bayhan H, Kir M. Clinical experience with Tc-99m MIBI imaging in patients with malignant tumors. Preliminary results and comparison with Tl-201. Clinical Nuclear Medicine 1992; 17: 171-6.

73. Campeau RJ, Kronemer KA, Sutherland CM. Concordant uptake of Tc-99m sestamibi and Tl-201 in unsuspected breast tumor. Clin Nucl Med 1992; 17(12): 936-7.

74. Del Vecchio S, Salvatore M. 99mTc-MIBI in the evaluation of breast cancer biology. European Journal of Nuclear Medicine and Molecular Imaging 2004; 31: S88-S96.

75. Brem RF, Floerke AC, Rapelyea JA, et al. Breast-specific gamma imaging as an adjunct imaging modality for the diagnosis of breast cancer. Radiology 2008; 247: 651-657.

76. Weber WP, Soysal SD, Fulco I, et al. Standardization of oncoplastic breast conserving surgery. Eur J Surg Oncol 2017; 43(7): 1236-43.

77. Yoon JJ, Green WR, Kim S, et al. Oncoplastic breast surgery in the setting of breast-conserving therapy: A systematic review. Adv Radiat Oncol 2016; 1(4): 205-15.

78. Castaneda SA, Strasser J. Updates in the Treatment of Breast Cancer with Radiotherapy. Surg Oncol Clin N Am 2017; 26(3): 371-82.

79. Brown LC, Mutter RW, Halyard MY. Benefits, risks, and safety of external beam radiation therapy for breast cancer. Int J Womens Health 2015; 7: 449-58.

80. Mondal, Sharma. External beam radiation techniques for breast cancer in the new millennium: New challenging perspectives. J Egypt NatlCanc Inst 2016; 28(4): 211-8.

81. Ades, Tryfonidis, Zardavas.The past and future of breast cancer treatment-from the papyrus to individualized treatment approaches. E cancer medical science 2017;11: 746.

82. Rapoport BL, Demetriou GS, Moodley SD, Benn CA. When and how do I use neoadjuvant chemotherapy for breast cancer? Curr Treat Options Oncol 2014; 15(1): 86-98.

83. Rubovszky G, Horváth Z. Recent Advances in the Neoadjuvant Treatment of Breast Cancer. J Breast Cancer 2017; 20(2): 119-31.

84. Rampurwala MM, Rocque GB, Burkard ME. Update on Adjuvant Chemotherapy for Early Breast Cancer. Breast Cancer (Auckl) 2014; 8: 125-33.

85. Anampa J, Makower J, Sparano JA. Progress in adjuvant chemotherapy for breast cancer: an overview. BMC Med 2015; 13: 195.

86. Rezvani K, Rource RH. The Application of Natural Killer Cell immunotherapy for the Treatment of Cancer. Frontiers in Immunology 2015; 6(578): 13.

87. Rastelli F, Crispino S. Factors predictive of response to hormone therapy in breast cancer. Tumori 2008; 94(3): 370-83.

88. Puhalla S, Bhattacharya S, Davidson NE. Hormonal therapy in breast cancer: a model disease for the personalization of cancer care. Mol Oncol 2012; 6(2): 222-36.

89. Slamon D, Eiermann W, Robert N, et al.; Breast Cancer International Research Group. Adjuvant trastuzumab in HER2-positive breast cancer. N Engl J Med 2011; 365(14):1273-83.

90. Johnston SR. Enhancing Endocrine Therapy for Hormone Receptor-Positive Advanced Breast Cancer: Cotargeting Signaling Pathways. J Natl Cancer Inst 2015;107 (10). pii:djv212.

91. Ma CX, Sanchez C, Gao F, et al. A Phase I Study of the AKT Inhibitor MK-2206 in Combination with Hormonal Therapy in Postmenopausal Women with Estrogen Receptor-Positive Metastatic Breast Cancer. Clin Cancer Res 2016; 22(11): 2650-8.

92. Yardley DA, Noguchi S, Pritchard KI, et al. Everolimus plus exemestane in postmenopausal patients with HR(+) breast cancer: BOLERO-2final progression-free survival analysis. Adv Ther 2013; 30(10): 870-84.

3 **MATERIAIS E EQUIPAMENTOS**

3.1 **Materiais**

Para realização deste trabalho foram utilizados os materiais descritos a seguir:

- ✓ Aptâmero Anti MUC 1, gentilmente sintetizado e doado pelo prof. Dr. Sotiris Missailidis do Instituto de Tecnologia em Imunobiológicos (Biomanguinhos), Fundação Oswaldo Cruz.
- ✓ Linhagem celulares MDA-MB-231 obtidas a partir da *American Type Culture Collection* (Manassas, VALLC), em cultura por RPMI pelo Thermo Fisher Scientific, MA USA;
- ✓ FBS (*Fetal Bovine Serum* – Soro Fetal Bovino) – Gibco, Life Technologies, MD, USA;
- ✓ Gentamicina – Gibco, Life technologies, MD, EUA;
- ✓ Diclorometano adquirido da VETEC®, grau de pureza P.A.;
- ✓ ^{99m}Tc (Tecnécio 99 metaestável) do IPEN/CNEN, fornecido pela Universidade Estadual do Rio de Janeiro (UERJ);
- ✓ $SnCl_2$ (Cloreto Estanoso) adquirido da Sigma Aldrich®, St Louis, MO, USA;
- ✓ Álcool polivinílico hidrolisado adquirido da Merck, grau de pureza P.A.;
- ✓ Acetona adquirido da Merck, grau de pureza P.A.;
- ✓ Álcool etílico adquirido da Merck, grau de pureza P.A.;
- ✓ Papel de *Whatmam* nº 1;
- ✓ Camundongos fêmeas BALB/c adquiridas do Biotério da Universidade de São Paulo (USP);
- ✓ Cetamina 10 g / 100 mL, comercializado pela Syntec®;
- ✓ Xilazina 2 g / 100 mL, comercializado pela Syntec®;

3.2 **Equipamentos**

No desenvolvimento deste trabalho foram utilizados os seguintes equipamentos:

- ✓ Balança Ay220, Shimadzu;
- ✓ Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK) – Laboratório de Desenvolvimento Galênico (LADEG) – Farmácia Universitária – Universidade Federal do Rio de Janeiro (UFRJ);
- ✓ Ultrasonicator UP100H (100 W, 30 kHz) (Hielscher, Teltow, Germany) – Laboratório de Desenvolvimento Galênico (LADEG) – Farmácia Universitária – Universidade Federal do Rio de Janeiro (UFRJ);
- ✓ Rotaevaporador (Heidolph, Schwabach, Germany) – Laboratório de Desenvolvimento Galênico (LADEG) – Farmácia Universitária – Universidade Federal do Rio de Janeiro (UFRJ);
- ✓ Contador gamma (Perkin Elmer Wizard® 2470, Perkin Elmer, Shelton, CT, USA) – Laboratório de Nanorradiofármacos – Associação Brasileira de Radiofarmácia;
- ✓ Câmara gamma Millenium (GE Healthcare, Cleveland, OH, USA) – Universidade Federal do Rio de Janeiro (UFRJ);
- ✓ Câmara de gás carbônico
- ✓ Cilindro de gás carbônico

CONSIDERAÇÕES FINAIS

A expressão aberrante de mucina pode estar associado ao crescimento do câncer, diferenciação, transformação e invasão (HOLLINGSWORTH and SWANSON, 2004). No câncer de mama, a maioria dos tumores expressam MUC1 e MUC3, diferente da expressão das isoformas MUC2, MUC4, MUC5AC e MUC6 que são variáveis ou limitadas (RAKHA et al., 2005).

KIM et al. (2009) descreveram duas estratégias básicas de sistemas de direcionamento a um alvo: 1) sinalização passiva, que age em consonância com o efeito de evidenciada permeabilidade e retenção (EPR) e 2) sinalização ativa, que emprega vetores ou ligantes direcionais. Utilizando-se desses conceitos, exploramos o aumento da permeabilidade vascular e a formação de espaços endoteliais para a drenagem de macromoléculas com tamanhos característicos (na faixa de 10 a 500 nm) para o interior dos tecidos (efeito EPR) em carcinogênese e aumentamos a eficiência do sistema de liberação ao associá-lo a ligantes alvo, no caso, a presença do anti-MUC1.

A incorporação de ligantes ativos, vetorização específica, na superfície de nanopartículas pode fomentar a interação dessas com células tumorais por um período de tempo apreciável, proporcionando o direcionamento e o reconhecimento de tumores ou revascularizações de forma ativa, o que pode melhor significativamente o índice terapêutico pela redução da toxicidade (MURPHY et al.; TIAN et al., 2011).

A técnica de polimerização por dupla emulsificação possibilitou sintetizar nanopartículas de PLGA de maneira satisfatória quanto à composição, possuindo distribuição de tamanho uniforme, perfil monomodal e baixo índice de polidispersividade. Também mostrou-se adequada para a preparação de nanopartículas com tamanho médio em torno dos 250nm.

As medidas de espalhamento dinâmico de luz na solução de NPs –AntiMUC1 foram utilizadas para medir o tamanho médio das nanopartículas, logo após a síntese, e também em função do tempo decorrido após a síntese. O ensaio foi realizado com cubeta de quartzo de 12mm2, em triplicata a 25°C e em ângulo de 173º. Nesta análise foi possível perceber discreto aumento no tamanho da nanopartícula que contém o aptâmero na sua superfície, porém essa dimensão não proporciona interferências negativas ao proposto.

O processo de marcação das nanopartículas de PLGA sem aptâmero e as contendo anti MUC 1 foi viabilizado pelo método direto da nanopartícula vazia e com o

oligonucleotídeo com o radionuclídeo ^{99m}Tc. Posteriormente, por cromatografia de camada delgada associada a contagem de energia gama foram avaliadas as estabilidades das NPs vazias (sem aptâmero) e cheias (com aptâmero) ligadas ao radionuclídeo, ambas apresentando, percentual superior a 99% em 8h de avaliação.

O modelo xenográfico de câncer de mama foi idealizado de maneira satisfatória pela injeção subcutânea da linhagem celular MDA-MB-231 no dorso de camundongos BALB/c. O crescimento de 2 cm da massa tumoral se deu após o período de 3 semanas e não foram evidenciados quadros clínicos de angústia, ascite, paralisia ou perda excessiva de peso nos camundongos, o que viabiliza-os para serem avaliados neste estudo.

O desempenho das Nps-PLGA-AntiMUC1-99mTc e Nps-PLGA-99mTc *in vivo* foram avaliadas a partir da injeção intraocular em camundongos BALB/c fêmeas (n=6 para cada grupo) para gerção de imagens cintilográficas em gama-câmara. Transcorridas 2h, os animais foram anestesiados e sacrificados, retirando-se sangue, cérebro, pulmões, estômago, fígado, coração, baço, rins, bexiga e lesão para a determinação da radioatividade em contador de radiação gama.

As nanopartículas de PLGA vazias mostraram alto grau hidrofílico, apresentando o clearence renal com taxa maior que 75% de toda atividade inicial. A contagem no fígado pode ser referenciada pelo efeito de opsonização ou eliminação rápida das nanopartículas presentes na circulação sanguínea pelo sistema fogocítico mononuclear (SFM), representado pelas células Kupffer do fígado e macrófagos do baço, conceito já explorado por autores como MOSQUEIRA et al., 2001 e SIBATA et al., 2004. No entanto, vale ressaltar que não houveram níveis detectáveis de radiação gama na lesão tumoral dos camundongos quando foram utilizadas as nanopartículas sem o aptâmero anti MUC 1.

Baseando-se no propósito de direcionamento das nanopartículas para a lesão, foi possível concluir que a atividade biológica apresentada pelas nanopartículas carregadas com oligonucleotídeo, anti-MUC1, e radiomarcadas com 99mTc foi altamente interativa com a superexpressão de MUC 1 na superfície da linhagem celular MDA-MB-231 presentes na lesão de câncer de mama no modelo xenográfico desenvolvido. Com isso, as Nps-PLGA-AntiMUC1-99mTc foram suficientemente encontradas no interstício do tumor sólido de mama por meio de imagens SPECT, com alta precisão do processo tumoral localizado no dorso dos camundongos BALB/c avaliados, não interagindo com tecidos não afetados pela tumorogênese ou apresentando níveis ínfimos de detecção, que não comprometem o estudo. Comparadas as nanopartículas vazias, as com o aptâmero mostram uma significativa detecção da radiação gama no tecido tumoral, mostrando as vantagens do uso da vetorização específica

e determinando o potencial uso do aptâmero anti-MUC1 radiomarcado ao radioisótopo emissor de radiação β, 99mTc, como agente de imagiologia molecular na área da medicina.

Todos os resultados acima descritos, além de se fazerem necessários para o desenvolvimento e aplicação de novos fármacos com fim diagnóstico, foram suficientes para a continuidade dos estudos *in vivo*. Diante do exposto, pode-se concluir que a nanotecnologia aplicada no desenvolvimento de nanoparticulas modificadas por aptâmero anti MUC 1 e radiomarcadas pelo 99mTc torna-se uma alternativa diagnóstica viável e de considerável importância, uma vez que se apresenta mais eficaz que a fórmula convencional de diagnóstico por estar associada a detecção em nível molecular, além de reduzir de maneira significativa o tempo para a introdução do tratamento. Portanto, os dados acima fornecem bases científicas suficientes para o desenvolvimento de novos estudos clínicos que possibilitarão a entrada no mercado dessa formulação nanoestruturada.

Sugestões para trabalhos futuros

Sugerimos como continuidade deste trabalho um estudo detalhado do processo de viabilidade celular, ensaios *in vitro*, para determinação da ocorrência ou não da toxicidade sobre linhagens tumorais, testadas para fins exclusivamente diagnósticos.

Propostas que avaliem o perfil de concentração plasmática das soluções farmacológicas de NPs utilizadas nos camundongos também são desejadas.

Estudos que extrapolem o tempo de geração de imagens SPECT nos camundongos resultando em ótima qualidade de análise.

Avaliar as características de outros polímeros, como PLA, associados ao aptâmero anti MUC 1 e 99mTc.

REFERÊNCIAS

Ades, Tryfonidis, Zardavas.The past and future of breast cancer treatment-from the papyrus to individualized treatment approaches. E cancer medical science 2017;11: 746.

Agboola AO, Banjo AA, Anunobi CC, Salami B, Agboola MD, Musa AA, Nolan CC, Rakha EA, Ellis IO, Green AR (2013) Cell Proliferation (KI-67) Expression is associated with poorer prognosis in Nigerian compared to British breast cancer women. ISRN Oncol 2013:675051. doi:10.1155/2013/675051

Akinyemiju TF, Pisu M, Waterbor JW, Altekruse SF. Socioeconomic status and incidence of breast cancer by hormone receptor subtype. Springerplus. 2015; 4:508. doi: 10.1186/s40064-015-1282-2 PMID: 26405628.

Aktolun C, Bayhan H, Kir M. Clinical experience with Tc-99m MIBI imaging in patients with malignant tumors. Preliminary results and comparison with Tl-201. Clinical Nuclear Medicine 1992; 17: 171-6.

Albernaz M de S, Ospina CA, Rossi AM, Santos-Oliveira R. Radiolabelled nano hydroxyapatite with 99mTc: perspectives to nano radiopharmaceuticals construction. Artif Cells Nanomed Biotechnol. 2014;42(2):88–91.

Altekruse, SF.; Kosary, CL.; Krapcho, M.; Neyman, N.; Aminou, R.; Waldron, W.; Ruhl, J.; Howlader, N.; Tatalovich, Z.; Cho, H.; Mariotto, A.; Eisner, MP.; Lewis, DR.; Cronin, K.; Chen, HS.; Feuer, EJ.; Stinchcomb, DG.; Edwards, BK., editors. SEER Cancer Statistics Review, 1975-2007, based on November 2009 SEER data submission, posted to the SEER web site. National Cancer Institute; Bethesda, MD: 2010. Available from: http://seer.cancer.gov/csr/1975_2007.

Amirikia KC, Mills P, Bush J, Newman LA. Higher populationbased incidence rates of triple-negative breast cancer among young African-American women: implications for breast cancer screening recommendations. Cancer. 2011;117:2747---53.

Anampa J, Makower J, Sparano JA. Progress in adjuvant chemotherapy for breast cancer: an overview. BMC Med 2015; 13: 195.

Andaya AA, Enewold L, Horner M- J, Jatoi I, Shriver CD, Zhu K. Socioeconomic disparities and breast cancer hormone receptor status. Cancer Causes Control. 2012 Jun; 23(6):951–8. doi: 10.1007/s10552- 012-9966-1 PMID: 22527173.

Anselmo AC, Gupta V, Zern BJ, et al. Delivering nanoparticles to lungs while avoiding liver and spleen through adsorption on red blood cells. ACS Nano. 2013;7(12):11129–11137.

Auguste A, Cortet M, Dabakuyo-Yonli TS, Launay L, Arnould L, Desmoulins I, Roignot P, Darut-Jouve A, Poillot ML, Bertaut A, Arveux P. Breast cancer subtype of French women is not influenced by socioeconomic status: A population-based-study. PLoS One. 2017 Feb 15;12(2):e0170069. doi: 10.1371/journal.pone.0170069. eCollection 2017.

Badve S, Dabbs DJ, Schnitt SJ, Baehner FL, Decker T, Eusebi V, Fox SB, Ichihara S, Jacquemier J, Lakhani SR, Palacios J, Rakha EA, Richardson AL, et al. Basallike and triple-negative breast cancers: a critical review with an emphasis on the implications for pathologists and oncologists. Modern Pathology. 2010; 24: 157-167.

Bae et al. Breast Density and Risk of Breast Cancer in Asian Women: A Meta-analysis of Observational Studies. J Prev Med Public Health. 2016 Nov;49(6):367-375.

Baek JS, Kim JH, Park JS, Cho CW. Modification of paclitaxel-loaded solid lipid nanoparticles with 2-hydroxypropyl-b-ciclodextrin enhances absorption and reduces nephrotoxicity associated with intravenous injection. Int J Nanomedicine. 2015;10:5397–5405.

Bandera EV, Maskarinec G, Romieu I, et al. Racial and Ethnic Disparities in the Impact of Obesity on Breast Cancer Risk and Survival: A Global Perspective. Advances in Nutrition 2015; 6(6): 803-19.

Banegas MP, Tao L, Altekruse S, Anderson WF, John EM, Clarke CA, et al. Heterogeneity of breast cancer subtypes and survival among Hispanic women with invasive breast cancer in California. Breast Cancer Res Treat. 2014 Apr; 144(3):625–34. doi: 10.1007/s10549-014-2882-1 PMID: 24658879.

Barbosa RCC, Da Costa DM, Ellen D, et al. Interaction of MTHFR C677T and A1298C, and MTR A2756G Gene Polymorphisms in Breast Cancer Risk in a Population in Northeast Brazil. Anticancer Research November 2012; 32(11): 4805-11.

Barcellos-Hoff MH, Lyden D, Wang TC. The evolution of the cancer niche during multistage carcinogenesis. Nature Reviews Cancer 2013; 13(7): 511-8.

Barnes Cj, Kumar R. Biology of the epidermal growth factor receptor family. Cancer Treat Res 2004; 119: 1-13.

Bauer KR, Brown M, Cress RD, Parise CA, Caggiano V. Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype: a population-based study from the California cancer Registry. Cancer. 2007 May 1;109(9):1721-8.

Blanco E, Shen H, Ferrari M. Nanoparticle rational design implementation for overcoming delivery barriers. Nat Biotechnol. 2015;33:941–951.

Borbas KE, Ferreira CS, Perkins A, Bruce JI, Missailidis S. Design and synthesis of mono- and multimeric targeted radiopharmaceuticals based on novel cyclen ligands coupled to anti-MUC1 aptamers for the diagnostic imaging and targeted radiotherapy of cancer. Bioconjug Chem. 2007;18(4):1205–1212.

Boyd NF, Martin LJ, Yaffe MJ, Minkin S. Mammographic density and breast cancer risk: current understanding and future prospects. Breast Cancer Res 2011;13(6):223.

Bozorgi A, Khazaei M, Khazaei MR. New Findings on Breast Cancer Stem Cells: A Review. J Breast Cancer 2015; 18(4): 303-12.

BRASIL. Ministério da Saúde. Secretaria de Atenção à Saúde. Instituto Nacional de Câncer. Coordenação de Prevenção e Vigilância. Estimativa 2016: Incidência de Câncer no Brasil. Rio de Janeiro, RJ, INCA, 2015.

BRASIL. Ministério da Saúde/SAS/INCA. Atlas de mortalidade por câncer no Brasil (2004–2014) Rio de Janeiro: INCA. Disponível em: https://mortalidade.inca.gov.br. Acesso em 10 de janeiro de 2016.

Brem RF, Floerke AC, Rapelyea JA, et al. Breast-specific gamma imaging as an adjunct imaging modality for the diagnosis of breast cancer. Radiology 2008; 247: 651-657.

Brenner DR, Brockton NT, Kotsopoulos J, et al. Breast cancer survival among young women: a review of the role of modifiable lifestyle factors. Cancer Causes Control 2016;27: 459-72. 56. Her J, Lee NS, Kim Y, et al. Factors forming the BRCA1-A complex orchestrate BRCA1 recruitment to the sites of DNA damage. Acta Biochimica et Biophysica Sinica 2016; 48(7): 658-64.

Brown LC, Mutter RW, Halyard MY. Benefits, risks, and safety of external beam radiation therapy for breast cancer. Int J Womens Health 2015; 7: 449-58.

Cadoo KA, Mcardle O, O'Shea AM, et al. Management of unusual histological types of breast cancer. Oncologist 2012; 17(9): 1135-45.

Campeau PM, Foulkes WD, Tischkowitz MD. Hereditary breast cancer: New genetic developments, new therapeutic avenues.Human Genetics 2008; 124(1):31–42.

Campeau RJ, Kronemer KA, Sutherland CM. Concordant uptake of Tc-99m sestamibi and Tl-201 in unsuspected breast tumor. Clin Nucl Med 1992; 17(12): 936-7.

Carey LA, Perou CM, Livasy CA. Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. JAMA. 2006;295(21):2492–502.

Castaneda SA, Strasser J. Updates in the Treatment of Breast Cancer with Radiotherapy. Surg Oncol Clin N Am 2017; 26(3): 371-82.
Chabalier C, Lamare C, Racca C, Privat M, Valette A, Larminat F. BRCA1 down regulation leads to premature inactivation of spindle checkpoint and confers paclitaxel resistance. Cell Cycle. 2006;5(9):1001–1007.

Chappuis P, Rosenblatt J, Foulkes W. The influence of familial and hereditary factors on the prognosis of breast cancer. Ann Oncol. 1999;10(10):1163–1170.

Chen L, Fan Y, Lang RG, et al. Breast carcinoma with micropapillary features: clinicopathologic study and long-term follow-up of 100 cases. Int J Surg Pathol 2008; 16: 155-63.

Chlebowski RT, Blackburn GL, Thomson CA, et al. Dietary fat reduction and breast cancer outcome: interim efficacy results from the Women's Intervention Nutrition Study. J Natl Cancer Inst. 2006;98:1767-1776.

Choi YE, Kwak JW, Park JW. Nanotechnology for early cancer detection. Sensors (Basel) 2010; 10(1): 428-55.

Chopra A, Shukla R, Sharma TK. Aptamers as an emerging player in biology. Aptamers Syn Antibodies. 2014;1(1):1–11.

Conroy et al. Racial/Ethnic Differences in the Impact of Neighborhood Social and Built Environment on Breast Cancer Risk: The Neighborhoods and Breast Cancer Study. Cancer Epidemiol Biomarkers Prev. 2017 Feb 14. pii: cebp.0935.2016. doi: 10.1158/1055-9965.

Corfield A, Myerscough N, Longman R, Sylvester P, Arul S, Pignatelli M. Mucins and mucosal protection in the gastrointestinal tract: news prospects for mucins in the phatology of gastrointestinal disease. Gut. 2000;47(4):589–594.

Da Pieve C, Blackshaw E, Missailidis S, Perkins AC. PEGylation and biodistribution of an anti-MUC1 aptamer in MCF-7 tumor-bearing mice. Bioconjug Chem. 2012;23(7):1377–1381.

Daley AJ, Crank H, Saxton JM, et al. Randomized trial of exercise therapy in women treated for breast cancer. J Clin Oncol. 2007;25:1713-1721.

Danforth DNJ. Disparities in breast cancer outcomes between Caucasian and African American women: a model for describing the relationship of biological and nonbiological factors. Breast Cancer Res. 2013; 15(3):208. doi: 10.1186/bcr3429 PMID: 23826992.

Delpu Y, Cordelier P, Cho WC, et al. DNA Methylation and Cancer Diagnosis. International Journal of Molecular Sciences 2013: 14(7): 15029-58.

De Abreu FB, Wells WA, Tsongalis GJ. The Emerging Role of the Molecular Diagnostics Laboratory in Breast Cancer Personalized Medicine. The American Journal Pathology 2013; 183(4): 1075-83.

Del Vecchio S, Salvatore M. 99mTc-MIBI in the evaluation of breast cancer biology. European Journal of Nuclear Medicine and Molecular Imaging 2004; 31: S88-S96.

DeSantis C, Ma J, Bryan L, Jemal A. Breast cancer statistics, 2013. CA Cancer J Clin. 2014;64(1):52–62.

Dietze EC, Sistrunk C, Miranda-Carboni G, O'Regan R, Seewaldt VL. Triple-negative breast cancer in African-American women: disparities versus biology. Nat Rev Cancer. 2015 Apr; 15(4):248–54. doi: 10. 1038/nrc3896 PMID: 25673085

Dong M, How T, Kirkbride KC, et al. The type IIITGF-β receptor suppresses breast cancer progression. J Clin Invest. 2007;117:206–217.

Easton DF. How many more breast cancer predisposition genes are there? Breast Cancer Research 1999; 1(1):14–17.

Eley JW, Hill HA, Chen VW, et al. Racial differences in survival from breast cancer: results of the National Cancer Institute Black/White Cancer Survival Study. JAMA. 1994;272:947-954.

Elmore JG, Armstrong K, Lehman CD, et al. Screening for Breast Cancer. The Journal of the American Medical Association 2005; 293(10): 1245-56.

Fejerman L, Romieu I, John EM et al. European ancestry is positively associated with breast cancer risk in Mexican women. Cancer Epidemiol Biomarkers Prev 2010;19:1074–1082.

Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015 Mar 1;136(5):E359-86. doi: 10.1002/ijc.29210.

Falco M, Palma G, Rea D, et al. Tumour biomarkers: homeostasis as a novel prognostic indicator. Open Biology 2016; 6(12): 11.

Feller L, Khammissa RAG, Lemmer J. Biomechanical cell regulatory networks as complex adaptive systems in relation to cancer. Cancer Cell International 2017; 17(16): 6.

Ferreira CS, Cheung MC, Missailidis S, Bisland S, Gariépy J. Phototoxic aptamers selectively enter and kill epithelial cancer cells. Nucleic Acids Res. 2009;37(3):866–876.

Ferreira CSM, Matthews C, Missailidis S. DNA aptamers that bind to Muc1 tumour marker: design and characterization of Muc1-binding single stranded DNA aptamers. Tumour Biol. 2006;27(6):289–301.

Ferreira CSM, Papamichael K, Guilbault G, Schwarzacher T, Gariepy J, Missailidis S. DNA aptamers against Muc1: design of aptamer-antibody sandwich ELISA for early tumour diagnosis. Anal Bioanal Chem. 2008;390(4):1618–2642.

Foulkes WD, Smith I, Reis-Filho JS. Triple-negative breast cancer. New England Journal of Medicine 2010; 363: 1938-48.

Fu D, Zuo Q, Huang Q, et al. Molecular Classification of Lobular Carcinoma of the Breast. Scientific Reports 2017: 7(43265).

Furberg H, Millikan R, Dressler L, Newman B, Geradts J. Tumor characteristics in African American and white women. Breast Cancer Res Treat. 2001;68: 33-43.

Gender SJ. MUC1, the renaissance molecule. J Mammary Gland Biol Neoplasia. 2001;6(3):339–353.

Gender SJ, Spicer AP. Epithelial mucin genes. Annu Rev Physiol. 1995; 57:607–634.

Girling A, Bartkova J, Burchell J, Gendler S, Gillett C, Taylor-Papadimitriou J. A core protein epitope of the polymorphic epithelial mucin detected by the monoclonal antibody SM-3 is selectively exposed in a range of primary carcinomas. Int J Cancer. 1989;43(6):1072–1076.

GLOBOCAN 2012: cancer incidence and mortality worldwide in 2012 – IARC; 2012. Available from: http://globocan.iarc.fr/Pages/fact_sheets_population.aspx. Accessed October 10, 2016.

Gold LS, Klein G, Kessler L, et al. The emergence of diagnostic imaging technologies in breast cancer: discovery, regulatory approval, reimbursement, and adoption in clinical guidelines. Cancer Imaging 2012; 12(1): 13-24.

Goss PE, Lee BL, Badovinac-Crnjevic T et al. Planning cancer control in Latin America and the Caribbean. Lancet Oncol 2013;14:391– 436.

Gouyer V, Dubuquoy L, Robbe-Masselot C, et al. Delivery of a mucin domain enriched in cysteine residues strengthens the intestinal mucous barrier. Sci Rep. 2015;15(5):9577.

Gutierrez C, Schiff R. HER2: biology, detection, and clinical implications. Arch Pathol Lab Med 2011; 135(1): 55-62.

Hamoudeh M, Kamleh MA, Diab R, Fessi H. Radionuclides delivery systems for nuclear imaging and radiotherapy of cancer. Adv Drug Deliv Rev. 2008;60:(12)1329–1346.

Hartwig FP, Nedel F, Collares T, et al. Oncogenic somatic events in tissue-specific stem cells: a role in cancer recurrence? Ageing Res Rev 2014; 13: 100-6.

Heywang-Köbrunner SH, Schreer I, Heindel W. Imaging Studies for the Early Detection of Breast Cancer. Deutsches Ärzteblatt International 2008; 105(31-32): 541-7.

Ho SB, Niehans GA, Lyftogt C, et al. Heterogeneity of mucin gene expression in normal and neoplastic tissues. Cancer Res. 1993;53:641–651.

Hollingsworth MA, Swanson BJ. Mucins in cancer: protection and control of the cell surface. Nat Rev Cancer. 2004;4(1):45–60.

Holmes MD, Chen WY, Feskanich D, et al. Physical activity and survival after breast cancer diagnosis. JAMA. 2005;293:2479-2486.

Hope KJ, Jin L, Dick JE. Acute myeloid leukemia originates from a hierarchy of leukemic stem cell classes that differ in self-renewal capacity. Nat Immunol 2004; 5(7): 738-43.

Howell A, Anderson AS, Clarke RB, et al. Risk determination and prevention of breast cancer. Breast Cancer Res 2014; 16: 446.

Howlader N, Noone AM, Krapcho M, et al. (eds). SEER Cancer Statistics Review, 1975-2011, National Cancer Institute. Bethesda, MD, http://seer.cancer.gov/csr/1975_2011/ , based on November 2013 SEER data submission, posted to the SEER web site, April 2014.

Hu Y, Duan J, Zhan Q, Wang F, Lu X, Yang XD. Novel MUC1 aptamer selectively delivers cytotoxic agent to cancer cells in vitro. PLoS One. 2012;7(2):e31970.

Hudis CA, Gianni L. Triple-negative breast cancer: an unmet medical need. Oncologist 2011; 16(Suppl. 1): 1–11. doi: 10.1634/ theoncologist.2011-S1-01.

Jeffrey SS, Fero MJ, Børresen-Dale AL, Botstein D. Expression array technology in the diagnosis and treatment of breast cancer. Mol Interv. 2002;2(2):101–109.
Jemal A, Center MM, Ward E, Thun MJ. Cancer occurrence. Methods Mol Biol. 2009;471:3–29.

Johnston SR. Enhancing Endocrine Therapy for Hormone Receptor-Positive Advanced Breast Cancer: Cotargeting Signaling Pathways. J Natl Cancer Inst 2015;107 (10). pii:djv212.

Justo et al. A review of breast cancer care and outcomes in Latin America. Oncologist. 2013;18(3):248-56. doi: 10.1634/theoncologist.2012-0373.

Kim GE, Bae HI, Park HU, et al. Aberrant expression of MUC5AC and MUC6 gastric mucins and sialylTn antigen in intraepithelial neoplasm of the pancreas. Gastroenterology. 2002;123(4):1052–1060.

Knobf MT, Ferrucci LM, Cartmel B, Jones BA, Stevens D, Smith M, et al. Needs assessment of cancer survivors in Connecticut. J Cancer Surviv. 2012;6(1):1–10.

Koch L, Jansen L, Brenner H, Arndt V. Fear of recurrence and disease progression in long-term (≥ 5 years) cancer survivorsz—a systematic review of quantitative studies. Psycho Oncol. 2013;22(1):1–11.

Kucia M, Ratajczak MZ. Stem cells as a two-edged sword-from regeneration to tumor formation. J Physiol Pharmacol 2006; 57(7): 5-16.

Kuroda H, Sakamoto G, Ohnisi K, et al. Clinical and pathologic features of invasive micropapillary carcinoma. Breast Cancer 2004; 11(2): 169-74.

Kushi LH, Doyle C, McCullough M, Rock CL, Demark-Wahnefried W, Bandera EV, Gapstur S, Patel AV, Andrews K, Gansler T, Society AC: Nutrition and Physical Activity Guidelines Advisory Committee: American Cancer Society Guidelines on nutrition and physical activity for cancer prevention: reducing the risk of cancer with healthy food choices and physical activity. CA Cancer J Clin 2010, 2012(62):30–67.

Land CE, Tokunaga M, Koyama K, et al. Incidence of female breast cancer among atomic bomb survivors, Hiroshima and Nagasaki, 1950-1990. Radiat Res. 2003; 160:707-717.

Lara-Medina F, Perez-Sanchez V, Saavedra-Perez D, et al. Triplenegative breast cancer in Hispanic patients: high prevalence, poor prognosis, and association with menopausal status, body mass index, and parity. Cancer. 2011;117:3658-69.

Lee MJ, Lee HS, Kim WH, Choi Y, Yang M. Expression of mucins and cytokeratins in primary carcinomas of the digestive system. Mod Pathol. 2003;16(5):403–410.

Lehman BD, Pietenpol J. Identifications and use of biomarkers in treatment strategies for triple negative breast cancers subtypes. Journal of Pathology 2014; 232: 142-50.

Lei L, Wang X, Chen Z. PET/CT Imaging for Monitoring Recurrence and Evaluating Response to Treatment in Breast Cancer. Advances in Clinical and Experimental Medicine 2016; 25(2): 377-82.

Liedtke, C., Hess, K.R., Karn, T., Rody, A., Kiesel, L., Hortobagyi, G.N., . . . Gonzalez-Angulo, A.M. (2013). The prognostic impact of age in patients with triple-negative breast cancer. Breast Cancer Research and Treatment, 138, 591–599. doi:10.1007/s10549-013 - 2461-x.

Lo P, Wolfson B, Zhou Q. Cancer stem cells and early stage basal-like breast cancer. World Journal of Obstetrics and Gynecology 2016; 5(2): 150-61.

Lv Q, Meng Z, Yu Y, et al. Molecular Mechanisms and Translational Therapies for Human Epidermal Receptor 2 Positive Breast Cancer. International Journal of Molecular Sciences 2016; 17(12).

Ma CX, Sanchez C, Gao F, et al. A Phase I Study of the AKT Inhibitor MK-2206 in Combination with Hormonal Therapy in Postmenopausal Women with Estrogen Receptor-Positive Metastatic Breast Cancer. Clin Cancer Res 2016; 22(11): 2650-8.

Maciejczyk A. New prognostic factors in breast cancer. Advances in Clinical and Experimental Medicine 2013; 22(1): 5-15.

Malhotra GK, Zhao X, Band H, et al. Histological, molecular and functional subtypes of breast cancers. Cancer Biology and Therapy 2010; 10(10): 955-60.

Martin LJ, Boyd NF. Mammographic density. Potential mechanisms of breast cancer risk associated with mammographic density: hypotheses based on epidemiological evidence. Breast Cancer Res 2008;10(1):201.

Matthews SB, Thompson HJ. The Obesity-Breast Cancer Conundrum: An Analysis of the Issues. International Journal of Molecular Sciences 2016; 17(6): 19.

Mehrgou A, Akouchekian M. The importance of BRCA1 and BRCA2 genes mutations in breast cancer development. The Medical Journal of the Islamic Republic of Iran 2016; 30(369): 12.

Mertz TM, Harcy V, Roberts SA. Risks at the DNA Replication Fork: Effects upon Carcinogenesis and Tumor Heterogeneity. Genes (Basel) 2017; 8(46): 21.
Mistry DAH, French PW. Circulating Phospholipids as Biomarkers of Breast Cancer: A Review. Breast Cancer 2016; 10: 191-6.

Mollard S, Fanciullino R, Giacometti S, et al. In Vivo Bioluminescence Tomography for Monitoring Breast Tumor Growth and Metastatic Spreading: Comparative Study and Mathematical Modeling. Scientific Reports 2016; 6: 10.

Mondal, Sharma. External beam radiation techniques for breast cancer in the new millennium: New challenging perspectives. J Egypt NatlCanc Inst 2016; 28(4): 211-8.

Morris GJ, et al. Differences in breast carcinoma characteristics in newly diagnosed African-American and Caucasian patients: a single-institution compilation compared with the National Cancer Institute's Surveillance, Epidemiology, and End Results database. Cancer. 2007;110(4):876–884.

Mouh FZ, Mzibri ME, Slaoui M and Amrani M. Recent Progress in Triple Negative Breast Cancer Research. Asian Pac J Cancer Prev. 2016; 17: 1595-1608.

Mosqueira, V.C.F. et al. Biodistribution of long-circulating PEG-grafted nanocapsules in mice: effects of PEG chain length and density. Pharmaceutical Research**,** v. 18, n.10, p. 1411-1419, 2001.

Murphy, E. A.; Majeti, B. K.; Mukthavaram, R.; Acevedo, l. M.; Barnes, l. A.; Cheresh, D. A. "Targeted Nanogels: A Versatile Platform for Drug Delivery to Tumors". Molecular Cancer Therapeutics 10(6), Junho de 2011.
Naqos N, Naim A, Jouhadi H, et al. Mucinous carcinoma of the breast: Clinical, biological and evolutive profile. Cancer Radiotherapie 2016; 20(8): 801-4.

Narayanan R, Dalton JT. Androgen Receptor: A Complex Therapeutic Target for Breast Cancer. Cancers 2016; 8(108):17.

Narod SA. Breast cancer in young women. Nat Rev Clin Oncol 2012;9:460– 470.

Nasir A, Shackelford RE, Anwar F, Yeatman TJ. Genetic risk of breast cancer. Minerva Endocrinol 2009;34:295–309.

Okubo Y, Okubo T, Okubo Y, et al. Neuroendocrine Differentiation in Breast Cancer: Clinicopathological Significance of Bcl-2 Positive Solid Papillary Carcinoma. Case Reports in Medicine 2016; 2016, ID 9501410: 6.

Pal T, Permuth-Wey J, Betts JA, et al. BRCA1 and BRCA2 mutations account for a large proportion of ovarian carcinoma cases.Cancer 2005; 104(12):2807–16.
Parise CA, Caggiano V. The Influence of Socioeconomic Status on Racial/Ethnic Disparities among the ER/PR/HER2 Breast Cancer Subtypes. J Cancer Epidemiol. 2015; 2015:813456. doi: 10.1155/2015/ 813456 PMID: 26339244.

Park SH, Kim MJ, Park BY, et al. Impact of Preoperative Ultrasonography and Fine-Needle Aspiration of Axillary Lymph Nodes on Surgical Management of Primary Breast Cancer. Annals of Surgical Oncology March 2011; 18(3):738-744.

Parsa Y, Mirmalek SA, Kani FE, et al. A Review of the Clinical Implications of Breast Cancer Biology. Electronic Physician 2016; 8(5): 2416-24.
Pettersson A, Graff RE, Ursin G, et al. Mammographic Density Phenotypes and Risk of Breast Cancer: A Metaanalysis. J Natl Cancer Inst 2014; 106(5): dju078.

Pierce JP, Natarajan L, Caan BJ, et al. Influence of a diet very high in vegetables, fruit, and fiber and low in fat on prognosis following treatment for breast cancer: the Women's Healthy Eating and Living (WHEL) randomized trial. JAMA. 2007;298:289-298.

Pieve CD, Perkins AC, Missailidis S. Anti-Muc1 aptamers: radiolabelling with (99m)Tc and biodistribution in Mcf-7 tumour-bearing mice. Nucl Med Biol. 2009;36(6):703–710.

Pinto SR, Sarcinelle MA, de Souza Albernaz M, et al. In vivo studies: comparing the administration via and the impact on the biodistribution of radiopharmaceuticals. Nucl Med Biol. 2014;41(9):772–774.

Porter PL, Lund MJ, Lin MG, et al. Racial differences in the expression of cell cycle-regulatory proteins in breast carcinoma. Cancer. 2004;100:2533- 2542.

Prat A, Parker JS, Karginava O, et al. Plenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. Breast Cancer Research 2010; 12: R68.

Prat A, Perou CM. Deconstructing the molecular portraits of breast cancer. Molecular Oncology 2011; 5: 5-23.

Puhalla S, Bhattacharya S, Davidson NE. Hormonal therapy in breast cancer: a model disease for the personalization of cancer care. Mol Oncol 2012; 6(2): 222-36.

Qian CN, Tan MH, Yang JP, et al. Revisiting tumor angiogenesis: vessel co-option, vessel remodeling, and cancer cellderived vasculature formation. Chinese Journal of Cancer 2016; 35(10): 6.

Rakha EA, Boyce RW, Abd El-Rehim D, Kurien T, Green AR, Paish EC, Robertson JF, Ellis IO. Expression of mucins (MUC1, MUC2, MUC3, MUC4, MUC5AC and MUC6) and their prognostic significance in human breast cancer. Mod Pathol. 2005;18(10):1295–304.

Rampurwala MM, Rocque GB, Burkard ME. Update on Adjuvant Chemotherapy for Early Breast Cancer. Breast Cancer (Auckl) 2014; 8: 125-33.

Rapoport BL, Demetriou GS, Moodley SD, Benn CA. When and how do I use neoadjuvant chemotherapy for breast cancer? Curr Treat Options Oncol 2014; 15(1): 86-98.

Rastelli F, Crispino S. Factors predictive of response to hormone therapy in breast cancer. Tumori 2008; 94(3): 370-83.

Reis-Filho JS, Tutt AN. Triple Negative Tumors: a Critical Review. Histopathology 2008; 52: 108-18.

Reis-Filho JS, Westbury C, Pierga JY. The impact of expression, profiling on prognostic and predictive testing in breast cancer. J Clin Pathol. 2006;59(3):225–231.

Ren Z, Li Y, Hameed O, et al. Prognostic factors in patients with metastatic breast cancer at the time of diagnosis. Pathology, Research and Practice 2014; 210(5): 301-6.

Reyna C, Lee MC (2014) Breast cancer in young women: special considerations in multidisciplinary care. J Multidiscip Healthc 29(7):419–429.

Rezvani K, Rource RH. The Application of Natural Killer Cell immunotherapy for the Treatment of Cancer. Frontiers in Immunology 2015; 6(578): 13.

Rivenbark AG, O'Connor SM, Coleman WB. Molecular and cellular heterogeneity in breast cancer. Challenges for personalized Medicine. American Journal of Pathology 2013; 183: 1113-24.

Robilotta Cecil C. A tomografia por emissçao de pósitrons: uma nova modalidade na medicina nuclear brasileira. Ver Panam Salud Publica [Positron emission tomography: a new modality in Brazilian nuclear medicine]. V 20, n. 2/3; 2006. Available from: http://journal.paho.org/uploads/1162234592.pdf. Acesso em 05 de março de 2016. Portuguese.

Rubovszky G, Horváth Z. Recent Advances in the Neoadjuvant Treatment of Breast Cancer. J Breast Cancer 2017; 20(2): 119-31.

Runowicz CD, Leach CR, Henry NL, Henry KS, Mackey HT, Cowens-Alvarado RL, Cannady RS, Pratt-Chapman ML, Edge SB, Jacobs LA, Hurria A, Marks LB, LaMonte SJ, Warner E, Lyman GH, Ganz PA. American Cancer Society/American Society of Clinical Oncology Breast Cancer Survivorship Care Guideline. CA Cancer J Clin. 2016 Jan-Feb;66(1):43-73. doi: 10.3322/caac.21319.

Sá LT, Simmons S, Missailidis S, da Silva MI, Santos-Oliveira R. Aptamer-based nanoparticles for cancer targeting. J Drug Target. 2013;21(5):427–434.

Sabatier R, Sabiani L, Zemmour C, et al. Invasive ductal breast carcinoma with predominant intraductal component: Clinicopathological features and prognosis. Breast 2016; 27:8-14.

Santos-Oliveira R. Nanoradiopharmaceuticals: is that the future for nuclear medicine? Curr Radiopharm. 2011;4(2):140–143.

Sarcinelli MA. Desenvolvimento de Nanopartícula de Poli(Ácido Lático) contendo Anticorpo Monoclonal marcada com 99mTc para o Diagnóstico Precoce e Tratamento Eficaz do Câncer de Mama [Tese de D.Sc]. Rio de Janeiro, RJ: IMA/UFRJ; 2014. Portuguese.

Schwartz TL, Mogal H, Papageorgiou C, et al. Metaplastic breast cancer: histologic characteristics, prognostic factors and systemic treatmentstrategies. Exp Hematol Oncol 2013; 2(1): 31.

Slamon D, Eiermann W, Robert N, et al.; Breast Cancer International Research Group. Adjuvant trastuzumab in HER2-positive breast cancer. N Engl J Med 2011; 365(14):1273-83.

Sengupta S, Pal S, Biswas BK, et al. Evaluation of Clinico-Radio-Pathological Features of Tubular Adenoma of Breast: a Study of Ten Cases with Histopathological Differential Diagnosis. Iranian Journal of Pathology 2015; 10(1): 17-22.

SIBATA, M.N. et al. Photophysicals and photochemicals studies of zinc (II) phtalocyanine in long time circulation micelles for photodynamic therapy. European Journal of Pharmaceutical Sciences, v. 23, p. 131 – 138, 2004.

Siegel, R., Miller, K. and Jemal, A. (2015) Cancer statistics, 2015. CA Cancer J Clin 65: 5–29.

Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin. 2016 Jan-Feb;66(1):7-30. doi: 10.3322/caac.21332. Epub 2016 Jan 7.

Sineshaw HM, Gaudet M, Ward EM, Flanders WD, Desantis C, Lin CC, et al. Association of race/ethnicity, socioeconomic status, and breast cancer subtypes in the National Cancer Data Base (2010–2011). Breast Cancer Res Treat. 2014 Jun; 145(3):753–63. doi: 10.1007/s10549-014-2976-9 PMID: 24794028.

Sinn HP, Kreipeb H. A Brief Overview of the WHO Classification of Breast Tumors, 4th Edition, Focusing on Issues and Updates from the 3rd Edition. Breast Care 2013; 8: 149-54.

Sorlie T. Molecular portraits of breast cancer: tumors subtypes as distinct disease entities. Eur J Cancer. 2004;40(18):2667–2675.

Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007; 131(5): 861-72.

Taylor-PapadimitriouJ, Burchell J, Miles DW, Dalziel M. MUC1 and cancer. Biochim. Biophys. Acta. 1999;1455(2–3):301–313.

TIAN, H.; TANG, Z.; ZHUANG, X.; CHEN, X.; JING, X.. "Biodegradable synthetic polymers: Preparation, functionalization and biomedical application". Progress in Polymer Science. 2011. doi: 10.1016/j.progpolymsci.2011.06.004.

Tong HV, Brindley PJ, Meyer CG, et al. Parasite Infection, Carcinogenesis and Human Malignancy. EBioMedicine 2017; 15: 12-23.

Tonin RS. Cintilografia e PET/CT nas neoplasias malignas [Scintigraphy and PET/CT in malignant neoplasms]. Porto Alegre, Rio Grande do Sul: UFRS; 2011. Portuguese.

Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015;65(2):87-108.

Torre LA, Siegel RL, Ward EM, Jemal A. Global Cancer Incidence and Mortality Rates and Trends--An Update. Cancer Epidemiol Biomarkers Prev. 2016 Jan;25(1):16-27. doi: 10.1158/1055-9965.EPI-15-0578. Epub 2015 Dec 14.

Tsutsui S, Ohno S, Murakami S, et al. Prognostic significance of the coexpression of p53 protein and c-erbB2 in breast cancer. Am J Surg 2003; 185(2): 165-7.

Venkitaraman A.R. Functions of BRCA1 and BRCA2 in the biological response to DNA damage. Journal of Cell Science 2001; 114(20): 3591-8.

Vercher-Conejero JL, Pelegrí-Martinez L, Lopez-Aznar D, et al. Positron Emission Tomography in Breast Cancer. Diagnostics 2015; 5(1): 61-83.

Viale G. The current state of breast cancer classification. Annals of Oncology 2012; 23 (Suppl 10): 207-10.

Villarreal-Garza et al. Breast cancer in young women in Latin America: an unmet, growing burden. Oncologist. 2013;18(12):1298-306. doi: 10.1634/theoncologist.2013-0321.

Vlad AM, Diaconu I, Gantt KR. MUC1 in endometriosis and ovarian cancer. Immunol Res. 2006;36(1–3):229–236.

Vona-Davis L, Rose DP. The influence of socioeconomic disparities on breast cancer tumor biology and prognosis: a review. J Womens Health (Larchmt). 2009 Jun; 18(6):883–93.

Wang XX, Jiang YZ, Liu XY, et al. Difference in characteristics and outcomes between medullary breast carcinoma and invasive ductal carcinoma: a population based study from SEER 18 database. Oncotarget 2016; 7(16): 22665-73.

Weber WP, Soysal SD, Fulco I, et al. Standardization of oncoplastic breast conserving surgery. Eur J Surg Oncol 2017; 43(7): 1236-43.

Wei S. Papillary Lesions of the Breast: An Update. Arch Pathol Lab Med 2016; 140(7): 628-43. doi: 10.5858/arpa. 2015-0092-RA. Review.

Wesoła M, Jeleń M. The Diagnostic Efficiency of Fine Needle Aspiration Biopsy in Breast Cancers – Review. Advances in Clinical and Experimental Medicine 2013; 22(6): 887-92.

Weston A, Harris CC. Multistage carcinogenesis. In Holland-Frei Cancer Medicine, 6th edition. B.C. Decker; 2003.

Willis RE. Targeted Cancer Therapy: Vital Oncogenes and a New Molecular Genetic Paradigm for Cancer Initiation Progression and Treatment. International Journal of Molecular Sciences 2016; 17(9): 23.

Yamashita T, Wang XW. Cancer stem cells in the development of liver cancer. J Clin Invest 2013; 123(5): 1911-8.

Yang YL, Liu BB, Zhang X, et al. Invasive micropapillary carcinoma of the breast: an update. Archives of Pathology and Laboratory Medicine 2016; 140(8): 799-805.

Yardley DA, Noguchi S, Pritchard KI, et al. Everolimus plus exemestane in postmenopausal patients with HR(+) breast cancer: BOLERO-2final progression-free survival analysis. Adv Ther 2013; 30(10): 870-84.

Ydiner A, Sen F, Tambas M, et al. Metaplastic Breast Carcinoma Versus Triple-Negative Breast Cancer. Survival and Response to Treatment. Medicine (Baltimore) 2015; 94(52): e2341.

Yoon JJ, Green WR, Kim S, et al. Oncoplastic breast surgery in the setting of breast-conserving therapy: A systematic review. Adv Radiat Oncol 2016; 1(4): 205-15.

Youlden DR, Cramb SM, Dunn NA, Muller JM, Pyke CM, Baade PD. The descriptive epidemiology of female breast cancer: an international comparison of screening, incidence, survival and mortality. CancerEpidemiol. 2012;36(3):237-48.

Yu C, Hu Y, Duan J, et al. Novel aptamer-nanoparticle bioconjugates enhances delivery of anticancer drug to MUC1-positive cancer cells in vitro. PloS One. 2011;6:e24077.

Yu J, Vodyanik MA, Smuga-Otto K. et al. Induced pluripotent stem cell lines derived from human somatic cells. Science 2007; 318(5858): 1917-20.

Yu K, Sang QXA, Lung PY, et al. Personalized chemotherapy selection for breast cancer using gene expression profiles. Scientific Reports 2017; 7(43294); 10.

Xu F, Liu F, Zhao H, An G, Feng G. Prognostic significance of MUC1 in various human epithelial cancer: a meta-analysis. Medicine. 2015;94(50):e2286.

Zhang YM, Liu N, Zhu ZH, Rusckowski M, Hnatowich DJ. Influence of different chelators (HYNIC, MAG3 and DTPA) on tumor cell accumulation and mouse biodistribution of technetium-99m labeled antisense DNA. Eur J Nucl Med. 2000;27(11):1700–1707.

Zhou J, Satheesan S, Li H, et al. Cell-specific RNA aptamer against human CCR5 specifically targets HIV-1 susceptible cells and inhibits HIV-1 infectivity. Chem Biol. 2015;22(3):379–390.

Zhou Z, Hick DG. HER2 - Amplification or Overexpression in Upper GI Tract and Breast Cancer with Clinical Diagnosis and Treatment. In Oncogene and Cancer – From Bench to Clinic. Ed. Yahwardiah Siregar, 2013.