Original Article

TRIPLE-NEGATIVE BREAST CANCER DOES NOT FULLY OVERLAP WITH "BASAL-LIKE" MOLECULAR PROFILE – A MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY

Savelina L. Popovska,
Akishi Ooi[1],
Ivan N. Ivanov,
Nina G. Ivanova[2],
Tereza B. Dineva

Department of Pathology,
Medical University-Pleven, Bulgaria
[1]Department of Molecular and Cellular Pathology,
Kanazawa University Graduate School of Medicine,
Kanazawa, Japan
[2]Department of Medical Oncology,
University Hospital,
Pleven, Bulgaria

Corresponding Author:
Savelina L. Popovska
Department of Pathology
Medical University-Pleven
1 St Kl.Ochridsky str.
Pleven, 5800
Bulgaria
e-mail: sapopovska@yahoo.com

Received: May 11, 2010
Revision received: June 03, 2010
Accepted: July 29, 2010

Summary

Breast cancer comprises a diverse group of diseases in terms ot clinical presentaion, morphology, molecular profile and response to therapy. Recent microarray studies indicated that breast cancer is a heterogeneous disease and may be divided into several distinct subtypes. It is not clear if the "triple-negative breast cancer" (TNBC) category corresponds completely to the basal-like group. The aim of the study was to identify the basal-like subtype of breast cancers among the group of TNBCs by examining their morphology and immunohistochemical characteristics. The majority of triple-negative cancers were high-grade invasive ductal carcinomas of no special type (NST)-72%, 22% were medullary breast carcinomas and 6% were others. Morphologically, TNBC were highly cellular tumors characterized by solid architecture with little or no tubule formation. Among 77 TNBCs using the five-marker method criteria for identification of basal-like breast cancers 62/77 (80%) were found positive for basal marker panel while 15/77 (20%) were negative for ER, PgR, Her2 and Cytokeratin 5/6 and EGFR as well. All 77 TNBCs are highly proliferative tumors showed high nuclear Ki-67 immunostaining. Although a significant overlap with basal-like carcinoma was observed, it seemed clear that 'triple negativity' should not be used as a surrogate marker for basal-like cancers.
Key words: breast cancer, triple negative breast cancer, immunohistochemistry

Introduction

Breast cancer comprises a diverse group of diseases in terms of clinical presentaion, morphology and histology, molecular profile, and response to therapy [1, 2].

Recent microarray studies have indicated that breast cancer is a heterogeneous disease and may be divided into several distinct and reproducible subtypes associated with different outcomes and prognosis.

Expression profiles have categorized invasive breast carcinomas into the following groups: luminal A and B subtypes (ER+/HER2-/+), HER2 + subgroup (ER-/HER2+), the basal-like subgroup (ER- and HER2-) characteristics of breast myoepithelial cells [3, 4].

Among the ones that have attracted the attention of investigators in recent years, is basal-like cancer. Tumors from this group have been found to be positive for expression of basal cytokeratins, and negative for oesrtogen receptor (ER) and HER 2.

The so-called "triple-negative breast cancer" TNBC is a term recently introduced in literature that refers to cancers not expressing ER, PgR, and HER 2 receptors [1, 5]. TNBCs are aggressive cancers that affect young women of low social status. [6].

According to J. A. Sparano et al there exist significant differences in gene expression between the TNBC and hormone receptor positive groups [7].

To date, it is not clear if the TNBC category corresponds completely to the basal-like group. However, patients with TNBC are clinically relevant because the tumors are more aggressive. Chemotherapy in these cases is the only treatment available because there are no specific molecular targets. TNBCs are also associated with later diagnosis and shorter survival. The normal female breast is a gland composed of branching ducts which originate from lobules and end in ducts at the nipple area.Normal breast ducts contain at least three types of epithelial cells: luminal cells, basal/myoepithelial cells, and progenitor cells [8].The basal cells are confined in a basal membrane which separates the luminal epitehial component from the specialized breast connective tissue and adipose tissue.

Myoepithelial and luminal epithelia can be distinguished by their different CK expression patterns. Myoepithelial cells typically express high-molecular weight cytokeratis such as CK 5/6, CK 14 and CK 17, while luminal cells typically express CK 8 and 18. Immunohisto-chemical studies for basal/myoepithelial and luminal CKs appear to be helpful in subtyping invasive breast carcinomas into distinct biological subtypes.

Basal/myoepithelial cytokeratins and other markers have been used to identify a subset of ER- and HER2-negative breast carcinomas that are associated with a poor prognosis, further supporting the idea that a basal-like phenotype exists [5-9, 10, 11, 12, 13].

However, there is currently no internationally recognized immunohistochemical panel to define basal-like breast cancer and several combinations of basal cytokeratins and myoepitehial markers have been proposed.

The aim of the investigation was to identify the basal –like subtype of breast cancers among the group of TNBCs by examining their morphology and immunohistochemical characteristics.

Patients and Methods

Archival, histological, formaline-fixed and paraffin-embedded materials from 77 female II and III stage breast cancer patients, verified in previous pathology reports as "triple- negative", were obtained in a retrospective study of the archives of the Department of Clinical Pathology, University Hospital-Pleven. Our study group was selected from a regional population database.

For that purpose a total of 513 clinical cases of breast cancer, treated surgically at the Department of Surgical Oncology, University Hospital - Pleven, during the period 2006-2009 were studied retrospectively.

The mean age of the studied group was 58 years-ranging from 36 to 78 years.

All H&E stained sections of these tumors were reviewed to confirm conventional morphological parameters according to the WHO classification 2003 by two pathologist (S.P and I.I) [2].

Histological grades were assigned using modified Elston & Ellis criteria [14].

All sections were immunohistochemically stained for ER, PgR, HER-2, epidermal growth factor receptor (EGFR), CK5/6 and Ki-67 according to protocols provided by the manufacturer (Table 1.).

CK5/6, and EGFR were chosen as they are established markers of basal/myoepithelial cells [15-17].

Cytokeratin 5/6 was scored positive if more than 5% (weak or strong) cytoplasmic and/or membranous invasive carcinoma cell staining was observed.

The Ki-67 positivity was quantified as percentage of positive nuclear staining of the tumor cells, 10% being a cut-off for active proliferation.

EGFR immunostaining was evaluated according to the FDA approved Dako EGFR PharmDX kit instruction. According to that, membrane reactivity above the background in more than 1% of tumor cells is considered a positive result.

Table 1. Antibody, Clone, Dilution and Manufacturer

Antibody	Clone	Dilution	Manufacturer
ER	Mouse AntiHuman 1D 5	1:1	DakoCytomation
PgR	PR-PgR 636 Mouse AntiHuman	1:1	DakoCytomation
HER-2	Polyclonal AntiHuman c-erb-B-2 Oncoprotein 1100	1:250	DakoCytomation
Ki-67	Anti-human Ki-67 Antigen Clone MIB-1	1:1	DakoCytomation
Cytokeratin 5/6	Anti-human Cytokeratin 5/6-clone D5/16 B4	1:50	Dako Cytomation
EGFR	Mouse monoclonal -clone 18C9 NOVO	1:100	DakoCytomation

Results

TNBCs were found to be rare – 77 cases (15% of breast cancers diagnosed and treated during the period 2006-2009). The majority of triple-negative cancers were high-grade invasive ductal carcinomas of no special type (NST) 72%, 22% were medullary breast carcinomas, and 6% were other types of carcinomas (Fig.1).

Morphologically, TNBC were highly cellular tumors characterized by solid architecture with little or no tubule formation. We found geographic necrosis in 40 cases (52%) of the TNBC, pushing margins in 44 cases (57%), and lymphocytic infiltrate in 33 cases (43%) (Fig. 2).

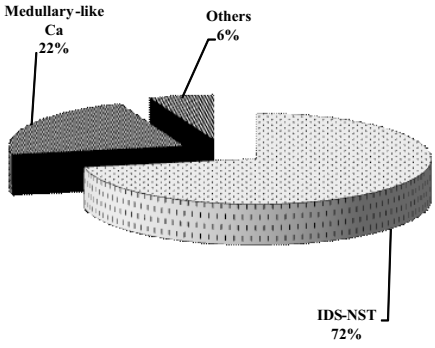

Fig. 1. Distribution of TNBT according their morphology

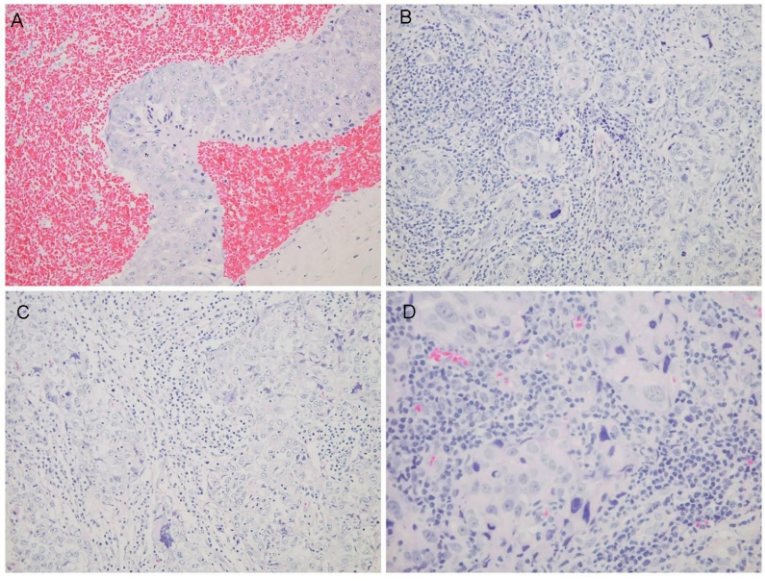

Fig. 2. A Ribbon -like architecture of invasive ductal carcinoma, areas of haemorrhages. Hematoxylin-eosin stain; original magnification, x100
B Medullar carcinoma of the breast –the tumor is composed of solid sheets of tumor cells, lack of tubule formation and lymphoplasmacytic infiltrate. Hematoxylin-eosin staining; original magnification, x100
C Grade III invasive ductal carcinoma of no special type IDC-NST or poorly differentiated invasive ductal carcinoma with high degree of pleomorphism and a moderate amount of mitoses. (original magnification x100
D Medullar carcinoma of the breast- synticial architecture and prominent nuclear pleomorphism. Hematoxylin-eosin staining; original magnification, x10.)

The nuclear chromatin pattern ranged from coarse to vesicular. Nucleoli were ranged from inconspicuous to prominent high histological grade and high mitotic index.

Of the 77 TNBCs, using the five-marker method criteria by Nielsen et al. [5] for identification of basal-like breast cancers, 62 of 77 (80%) were found positive for basal marker panel. The remainder 15 of 77 (20%) were negative for ER, PgR, Her2, as well as for Cytokeratin 5/6 and EGFR (Fig. 3).

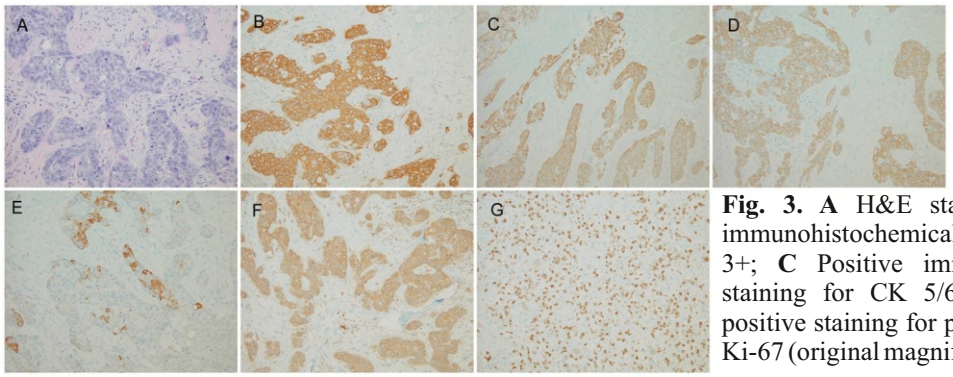

Fig. 3. A H&E staining; **B** Positive immunohistochemical staining for EGFR 3+; **C** Positive immunohistochemical staining for CK 5/6; **D** 90% nuclear positive staining for proliferation marker Ki-67 (original magnification x 100)

Of the 77 TNBCs, 58 (75%) of the cases were found with 90% or more tumor cell nuclei intensively stained, when tested with Ki-67. Seventeen (22%) cases showed Ki-67 nuclear immunostaining between 50-60%. In only two cases (2.6%) the stained nuclei were under 30% of all nuclei examined.

Discussion

In our study, the group of triple-negative cancers was heterogeneous. Most of the tumors were found to be high grade IDC NST, or medullary carcinoma.

Despite the great interest in basal-like cancers, there is still no internationally accepted definition of these tumours.

From a scientific point of view, microarray-based expression profiling analysis remains the "golden standard" for identification of basal-like breast cancers.

This technology is unlikely to be introduced in daily routine diagnostic practise in the foreseeable future, and results of microarray-based expression profiling using RNA extracted from formalin-fixed archival samples are suboptimal.

Several attempts to define an immunohisto-chemical surrogate for basal-like cancers have been described. The best example to date is the panel proposed by Nielsen et al., where basal-like cancers are defined as those lacking both ER and HER2 expression and expressing CK5/6 and/or EGFR. This panel has a specificity of 100% and a sensitivity of 76% for the identification of basal-like cancers [5].

Using the triple negative phenotype (TNP) method, basal-like carcinomas were negative for all routinely tested biomarkers: ER, PR, and HER2 (ER–PR–HER2–), and this surrogate definition of basal-like was referred to as TNP.

Using the five-biomarker method, TNP were divided into two groups: triple-negative cases (ER–, PR–, HER2–), which were also positively expressed for either EGFR or CK5/6. These cases were referred to as Core Basal TNBCs.

There was a five-marker negative phenotype (5NP), which was triple negative, and furthermore expressed neither EGFR nor CK5/6.

EGFR expression has been investigated in groups of triple-negative tumours, and was found to vary between 56% and 84% [18-19].

Patients with triple-negative cancers expressing a basal phenotype have been reported to have a significantly shorter disease-free survival, as compared with those with triple-negative cancers lacking the expression of basal markers. This is the reason why it is important to stratify patients with TNBCs [20].

EGFR activating gene mutations are remarkably rare. EGFR gene amplification has been shown in up to 25% of cases of metaplastic breast cancers - a subgroup of tumours that consistently show a triple-negative/basal-like phenotype [21].

A meta-analysis of more than 5000 breast cancer patients from 40 different series [22] has reported EGFR expression in 48% of the cases, using diferent techniques (range 14-91%).

In our study, positive staining for EGFR was

found in 57 of the 77 tumors (74%). Our results provide strong evidence that the use of five biomarker surrogate (ER, PgR, HER 2 EGRF and Ck 5/6) to define the basal-like subtype of breast cancer is significant for prognostication and proper clinical trial design.

Conclusion

The term triple-negative breast cancer encompasses a heterogeneous group of tumours that possess distinctive yet rather heterogeneous pathological and clinical features.

Although a significant overlap with basal-like carcinoma was observed, it seems that 'triple negativity' should not be used as a surrogate marker for basal-like cancers.

The development of new drugs and targeted therapies for triple-negative cancers is of paramount importance and requires better understanding of the complexity of this heterogeneous group of tumours.

By adding EGFR and CK5/6 as positive markers to TNP, a significantly worse outcome group can be identified among triple-negative cases. The Core Basal definition is associated with even poorer breast cancer survival in the whole population-based group and this high risk group may benefit from a more aggressive chemotherapy.

Acknowledgment

This research project was financially supported by MU-Pleven research project program 2009.

References

1. Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffreyk SS, Rees CA, et al. Molecular portraits of human breast tumours. Nature. 2000; 406(6797):747-52
2. Tavassoli FA, Devillee P. WHO Classification of Tumours: Pathology and Genetics of Tumours of the Breast and Female Genital Organs, Lyon: IARC Press; 2003. p. 9-110
3. Kreike B, van Kouwenhove M, Horlings H, Weigelt B, Bartelink H, van de Vijver MJ. Gene expression profiling and histopathological characterization of triple-negative/basal-like breast carcinomas. Breast Cancer Res. 2007;9:R65
4. Hu Z, Fan C, Oh DS, Marron JS, He X, Qaqish BF et al. The molecular portraits of breast tumors are conserved across microarray platforms. BMC Genomics. 2006;7:96
5. Nielsen TO, Hsu FD, Jensen K Cheang M, Karaca G, Hu Z, Hernandez-Boussard T et al. Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. Clin Cancer Res. 2004;10:5367-5374
6. Kanga SP, Martelb M, Harris LN. Triple negative breast cancer: current understanding of biology and treatment options. Curr Opin Obstet Gynecol. 2008;20:40-46
7. Sparano A, Goldestin LJ, Childs BH, Shak S, Badve S, Baehner FL et al. Genotypic characterization of phenotypically defined triple-negative breast cancer. J Clin Oncol. 2009; 27(15S):500
8. Adriance MC, Inman JL, Petersen OW, Bissell MJ. Myoepithelial cells: good fences make good neighbors. Breast Cancer Res. 2005;7:190-197
9. Abd El-Rehim DM, Pinder SE, Paish CE, Bell J, Blamey RW, Robertson JF et al. Expression of luminal and basal cytokeratins in human breast carcinoma. J Pathol. 2004;203:661-671
10. Kesse-Adu R, Shousha S. Myoepithelial markers are expressed in at least 29% of oestrogen receptor negative invasive breast carcinoma. Mod Pathol. 2004;17:646-652
11. Korsching E, Packeisen J, Agelopoulous K, et al. Cytogenetic alterations and cytokeratin expression patterns in breast cancer: integrating a new model of breast differentiation into cytogenetic pathways of breast carcinogenesis. Lab Invest. 2002;82:1525-1533
12. Tsuda H, Takarabe T, Hasegawa T, Murata T, Hirohashi S. Myoepithelial differentiation in high-grade invasive ductal carcinomas with large central acellular zones. Hum Pathol. 1999; 10:1134-1139
13. Tsuda H, Takarabe T, Hasegawa F, Fukutomi T, Hirohashi S. Large, central acellular zones indicating myoepithelial tumor differentiaton in high-grade invasive ductal carcinomas as markers of predisposition to lung and brain metastases. Am J Surg Pathol. 2000;24:197-202
14. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology. 1991;19(5):403-410
15. Heatley M, Maxwell P, Whiteside C, Toner P. Cytokeratin intermediate filament expression in benign and malignant breast disease. J Clin Pathol. 1995;48:26-32
16. van de Rijn M, Perou CM, Tibshirani R, Haas P, Kallioniemi O, Kononen J et al. Expression of cytokeratins 17 and 5 identifies a group of breast carcinomas with poor clinical outcome. Am J Pathol. 2002;161:1991-1996
17. Santini D, Ceccarelli C, Tardio ML, Taffurelli M,

Marrano D. Immunocytochemical expression of epidermal growth factor receptor in myoepithelial cells of the breast. Appl Immunohistochem Mol Morphol. 2002;10:29-33

18. Rakha EA, El-Sayed ME, Green AR Lee AH, Robertson JF, Ellis IO et al. Prognostic markers in triple-negative breast cancer. Cancer. 2007; 109;25-32
19. Tischkowitz M, Brunet JS, Begin LR Huntsman DG, Cheang MC, Akslen LA et al. Use of immunohistochemical markers can refine prognosis in triple negative breast cancer. BMC Cancer 2007;7:134
20. Tan DS, Marchio C, Jones RL, Savage K, Smith IE, Dowsett M et al. Triple negative breast cancer: molecular profiling and prognostic impact in adjuvant anthracycline-treated patients. Breast Cancer Res Treat. 2008;111:27-44
21. Reis-Filho JS, Pinheiro C, Lambros MB Milanezi F, Carvalho S, Savage K et al. EGFR amplification and lack of activating mutations in metaplastic breast carcinomas. J Pathol. 2006; 209:445-453
22. Klijn JG, Berns PM, Schmitz PI, Foekens JA. The clinical significance of epidermal growth factor receptor (EGF-R) in human breast cancer: A review on 5232 patients. Endocr Rev. 1992;13(1):3-17