Exp Oncol 2017
39, 1, 65–68

Experimental ONCOLOGY

SIGNIFICANCE OF IODINE SYMPORTER FOR PROGNOSIS OF THE DISEASE COURSE AND EFFICACY OF NEOADJUVANT CHEMOTHERAPY IN PATIENTS WITH BREAST CANCER OF LUMINAL AND BASAL SUBTYPES

*V.F. Chekhun[1, *], A.V. Andriiv[2, 3], N.Yu. Lukianova[1]*

[1]*R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine, Kyiv 03022, Ukraine*
[2]*Ivano-Frankivsk National Medical University, Ivano-Frankivsk 76018, Ukraine*
[3]*Precarpathian Clinical Oncology Center, Ivano-Frankivsk 76018, Ukraine*

The aim **of the research was to study the relation between expression of Na^+/I^- symporter (NIS) in breast cancer (BC) of different molecular subtypes and sensitivity of BC cells to neoadjuvant chemotherapy (NACT) and to assess whether NIS expression may be used as a predictive marker of treatment efficacy.** ***Materials and Methods*****: The study included 148 women with BC of stage II–III who were treated at the Precarpathian Clinical Oncology Center during 2012–2017. All patients were treated with NACT that included 2–6 cycles of chemotherapy by FAC, AC scheme with 21 day intervals. NACT efficacy was evaluated every 2 cycles by mammography according to RECIST criteria. Morphological and immunohistochemical study of NIS expression was performed by the standard methods on paraffin sections of surgically resected tumors.** ***Results*****: The heterogeneity of different molecular BC subtypes regarding response to the NACT has been found. Her2/neu-positive and basal BC subtypes were the least susceptible to the NACT ($p < 0.05$). It was shown that NIS expression is related to the sensitivity of luminal B and basal BC subtypes to the NACT. The highest expression of NIS and impairment of its functional activity was registered in the group of patients with tumors resistant to NACT (stabilization of the disease or its progression) of luminal B (220 ± 8.6 points) and basal subtypes (290 ± 11.3 points) ($p < 0.05$). It was revealed that the disease-free survival of patients with BC of luminal B and basal subtypes was higher in the absence of NIS expression in tumor cells ($p < 0.05$).** ***Conclusions*****: The results indicate that NIS can be used as an objective criterion for predicting the sensitivity of luminal B and basal BC subtypes to NACT, which will provide improved treatment outcomes in this group of patients.**
Key Words**: breast cancer, molecular subtypes, Na^+/I^- symporter, neoadjuvant chemotherapy.**

Breast cancer (BC) is the urgent problem of oncology because of high rates of morbidity in female population of many countries, including Ukraine [1]. In recent years, the prognosis of its clinical course has been based on determining the molecular subtypes of BC, namely luminal A, luminal B, basal or triple negative, HER2/neu-positive. It is accepted that these subtypes are characterized by different response to therapy, disease course and prognosis [2, 3].

Chemotherapy is one of the main treatments of BC patients used as a component of a combination therapy in neoadjuvant and/or adjuvant regimes. Chemotherapy is of particular importance for treatment of luminal B and triple-negative (basal) BC subtypes, since these tumors are characterized by high proliferative activity and low survival rates of the patients [4, 5]. A significant barrier to effective treatment of BC, which globally is considered one of the most serious problems of modern oncology, is a natural or acquired resistance of tumors to chemotherapeutic drugs [6, 7]. Presently in clinical practice a number of prognostic indexes is used, but to date there are no universally accepted objective markers to predict an efficacy of neoadjuvant chemotherapy (NACT) in treating BC of different molecular subtypes. Thus, the problem of predicting the effectiveness of NACT in the treatment of BC patients is the lack of specific markers of sensitivity of tumors to the existing anticancer drugs.

In recent years, there have been the reports evidencing that the development of hormone-dependent malignancies, including BC may be associated with impaired functional activity of the protein involved in the processes of iodine uptake by the cells: Na^+/I^- symporter (NIS). Under physiological conditions, this protein is expressed on the membrane of follicular cells of the thyroid gland [8, 9]. When this protein is translocated from the membrane to the cytoplasm, which is observed in certain pathological conditions, it loses its functional activity and does not provide iodine absorption by the cells from the microcirculation [10]. According to the data of the literature [8, 9], under normal state and conditions of physiological balance in the cells of the mammary gland NIS expression is absent. It is shown that NIS expression is observed in more than 50% of malignant breast tumors [11]. There are data on the correlation between NIS expression with some biological features of malignant breast tumors such as receptor status and proliferative activity [12]. Proofs of the latter are the results of our previous studies, according to which the most high rates of NIS expression and its malfunction is determined in cells with a high degree of malignancy and low sensitivity to doxorubicin [13]. The above mentioned data allow consider that it is reasonable to study NIS expression in tumor cells of patients with BC of different molecular subtypes to clarify its impact in drug resistance.

Submitted: February 18, 2017.
*Correspondence: E-mail: chekhun@onconet.kiev.ua
Abbreviations used: BC – breast cancer; CR – complete regression; NACT – neoadjuvant chemotherapy; NIS – Na^+/I^- symporter; PR – partial regression.

The aim of the research was to study the relation between expression of NIS and sensitivity of BC of different molecular subtypes to NACT and to assess whether NIS expression may be used as a predictive marker of treatment efficacy.

MATERIALS AND METHODS

Patients. The study included 148 women with BC stage II–III treated at the Precarpathian Clinical Oncology Center in 2012–2017. Tumor stage was determined according to the TNM classification (6th edition, 2002). The histological type of the resected tumors was verified upon morphological study (hematoxylin and eosin staining) according to the International Histological Classification of the World Health Organization (2006). All patients were treated with NACT. The course included 2–6 cycles of chemotherapy by FAC, AC scheme with 21 day-intervals. NACT efficacy was evaluated every 2 cycles by mammography according to RECIST criteria [14, 15]. All patients were informed and agreed to the use of biopsy material for research purposes.

Immunohistochemical study. Expression of NIS, estrogen receptors, progesterone, and proliferative activity marker (Ki-67) in tumor cells was studied on paraffin sections (4–5 microns) of biopsy material. As primary antibodies, mAbs specific for NIS (clone MA5–12308; Thermo Scientific, USA), estrogen receptor (clone 1D5), progesterone receptor (clone PgR636) and Ki-67 (MIB-1 clone; DakoCytomation, Denmark) were used. To visualize the reaction, EnVision System kit (Dako LSAB2 system, Denmark) was used according to the manufacturer's recommendations. The sections were stained with Mayer's hematoxylin. The expression of NIS was evaluated by semiquantitative method. Analysis of the results was performed using optical microscopy (× 100, oil immersion) using the classical method of H-Score [16]:

$$S = 1 \cdot N1^{+} + 2 \cdot N2^{+} + 3 \cdot N3^{+},$$

where S is H-Score index; $N1^{+}$, $N2^{+}$, $N3^{+}$ — number of cells with low, medium and high expression. The end result of the calculation is expressed in points: 50–100 points — low expression; 101–200 — medium expression; 201–300 points — high expression of NIS.

Statistical analysis. For the statistical analysis of the results of immunohistochemical studies, Statistica 6.0 program was used. To assess the significance of the differences between the expression of markers and other clinical and pathological parameters Student's *t*-test was used. Assessment of survival was determined by Kaplan — Meier method. The difference between the curves was assessed using the log-rank-test. The critical level of statistical significance was accepted at $p < 0.05$.

RESULTS AND DISCUSSION

The clinical characteristics of 148 patients with BC of stage II–III are shown in Table 1. According to the clinical data, the age of patients ranged from 28 to 72 years, mean age was 51.2 ± 6.4 years. The majority of patients (56.0%) were at menopause, menstrual function was preserved in 44.0% of patients. The number of patients with BC of stage II was 67 (45.3%), BC of stage III — 81 patients (54.7%). Upon comprehensive examination (X-ray, ultrasound, laboratory) conducted before treatment, metastases (N1–3) in regional lymph nodes were found in 113 patients (76.3%).

Table 1. Clinical characteristics of BC patients stage II–III

Index	Number of patients	
	n	%
Total number of patients	148	100
Age of patients (years)		
Average	51.2 ± 6.4	
Range	28–72	
Menstrual function		
Preserved	65	44.0
Menopause	83	56.0
BC stage by TNM		
II	67	45.3
III	81	54.7
Metastases in regional lymph nodes (category N)		
N0	35	23.7
N1–3	113	76.3
Morphology of BC		
Infiltrative ductal carcinoma	111	75.0
Infiltrative lobular cancer	37	25.0
Differentiation grade of BC		
G1 (high)	37	25.0
G2 (moderate)	72	48.6
G3 (low)	39	26.4
Molecular subtype of BC		
Luminal A	68	45.9
Luminal B	32	21.6
Her2/neu-positive	14	9.5
Basal	34	23.0

The distribution of patients by histological type of BC showed that most patients had infiltrative ductal carcinoma (75.0%) of moderate differentiation (48.6%). The greatest incidence was registered for luminal A subtype — 45.9%. Incidence of luminal B, Her2/neu-positive and basal subtypes of BC was 21.6; 9.5 and 23.0% respectively.

Depending on the degree of clinical effect of NACT (according to RECIST criteria) all patients were distributed into 2 groups. The 1st group included 71 BC patients who have demonstrated a positive response to the NACT: complete regression (CR) was observed in 11 patients, partial regression (PR) — in 60 patients. 2nd group was formed of 77 women with BC resistant to the treatment, including 56 patients with stabilization of tumor growth and 21 patients with BC progression in the setting of NACT (Table 2).

Analysis of the treatment effectiveness among different molecular BC subtypes has revealed their heterogeneity regarding their response to the NACT. According to the data given in Table 2, most frequently the positive effect of the treatment was detected in patients with luminal A subtype: in 79.4% cases positive effect (CR (16.2%) or PR (63.2%)) was observed. In the group of BC patients with luminal B subtype stabilization of tumor growth was observed in 71.9% cases and PR after the NACT — in 25% cases (Table 2). Her2/neu-positive and basal subtypes of BC showed the lowest sensitivity to NACT. As can be seen from the data (Table 2), PR was observed only in 14.2 and 20.6% of the investigated cases of Her2/neu-positive and basal BC subtypes, while in the rest of cases stabilization of the tumor growth or tumor progression were registered, indicating that NACT was ineffective (Table 2).

Table 2. Efficacy of NCT in BC of different molecular subtypes

Molecular subtype of BC	Number of patients, n (%)			
	The clinical effect of NACT (the RECIST criteria)			
	CR	PR	Stabilization	Progression
Luminal A (n = 68)	11 (16.2)	43 (63.2)*	14 (20.6)	0
Luminal B (n = 32)	0	8 (25.0)	23 (71.9)*	1 (3.1)
Her2/neu-positive (n = 14)	0	2 (14.2)	6 (42.9)	6 (42.9)*
Basal (n = 34)	0	7 (20.6)	13 (38.2)	14 (41.2)*

Note: *$p < 0.05$ compared with other molecular subtypes.

At the next stage of the study, we have analyzed clinical effect of NACT depending on the level of NIS expression in BC (Fig. 1). As shown in Fig. 2, in the tissue of luminal A and Her2/neu-positive subtypes either sensitive or resistant to NACT, expression of NIS was low (close to 100 points) compared with tumors of other molecular subtypes. In most tumors of BC patients with luminal B subtype who showed response to treatment, medium level of NIS expression (160 ± 7.8 points) was registered, while in BC patients with luminal B subtype who showed disease stabilization or tumor progression high level of NIS expression (220 ± 8.6 points) was detected ($p < 0.05$). The highest expression of NIS in tumor tissue was registered in the group of patients with basal BC ($p < 0.05$) (Fig. 2). Interestingly, in patients with basal BC subtype and the positive effect of the treatment the expression of NIS was 240 ± 7.6, while in patients with tumors resistant to NACT this index amounted to 290 ± 11.3 points ($p < 0.05$).

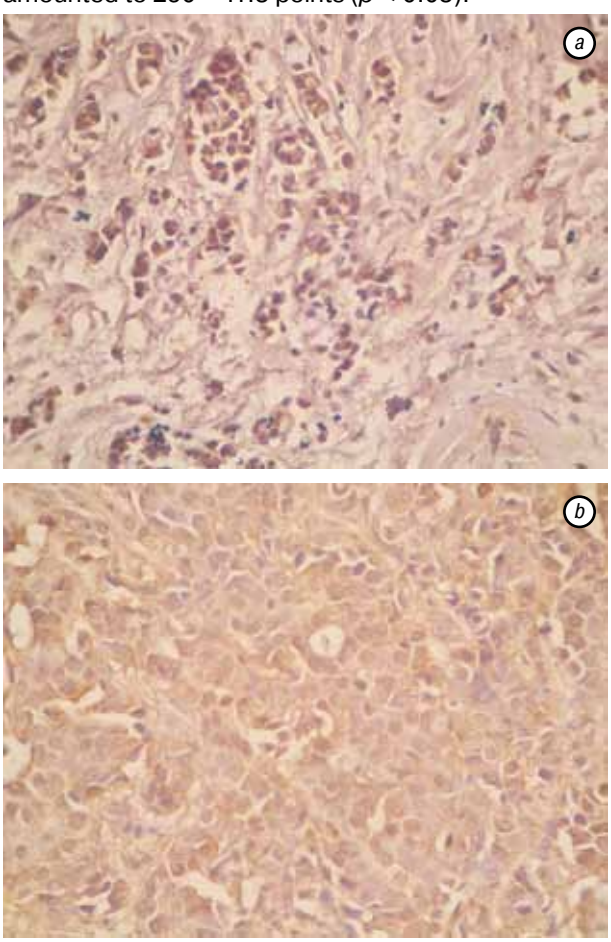

Fig. 1. Medium and high level of NIS expression in BC cells, immunohistochemical reaction, Meyer hematoxylin staining × 400

Since it is known that the NIS is capable to transport iodine only if it is expressed on the membranes of cells [10], we examined the localization of this protein in the tissue of BC of different molecular subtypes and with different sensitivity to the NACT. As show the data presented in Fig. 3, in the cells of luminal A and Her2/neu-positive BC subtypes NIS was expressed both on the membrane and in the cytoplasm, whereas in cells of luminal B and basal BC subtypes its localization was predominantly cytoplasmic ($p < 0.05$) (Fig. 3). The number of cells with membrane staining of NIS in luminal B and basal subtypes was 75 and 85%, respectively.

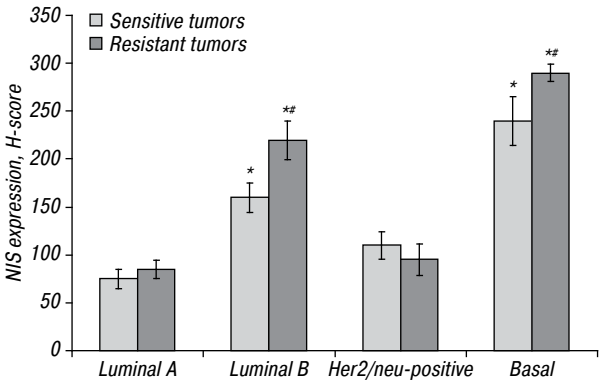

Fig. 2. The level of NIS expression in tumor cells of patients with BC of different molecular subtypes depending on the clinical effect of NCT. *$p < 0.05$ compared with other molecular subtypes; #$p < 0.05$ compared with sensitive tumors

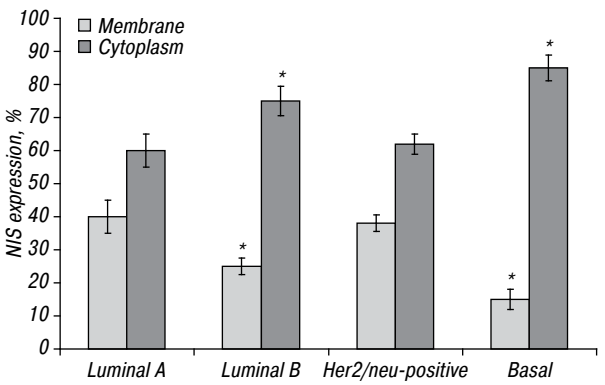

Fig. 3. Distribution of NIS expression in cells of different molecular subtypes of BC. *$p < 0.05$ compared with other molecular subtypes

As far as NIS expression could be related to the sensitivity of luminal B and basal subtypes to the NACT, we analyzed the survival of patients considering the presence of this protein in tumor cells. It was shown (Fig. 4) that the disease-free survival of patients with BC of luminal B and basal subtypes was higher in the absence of NIS expression in tumor cells. Relapse in patients with BC of luminal B subtype positive for NIS expression was determined in 12% of patients, while in patients with basal subtype — in 31% cases.

Thus, the present study has shown a relation between NIS expression and response to the NACT in patients with BC of certain molecular subtypes. The highest expression of NIS and disturbance of its functional activity was recorded in cells of luminal B and basal subtypes resistant to NACT. It was established that disease-free survival of patients with luminal B and basal BC subtypes is significantly more worse in the case of NIS expression in tumor cells.

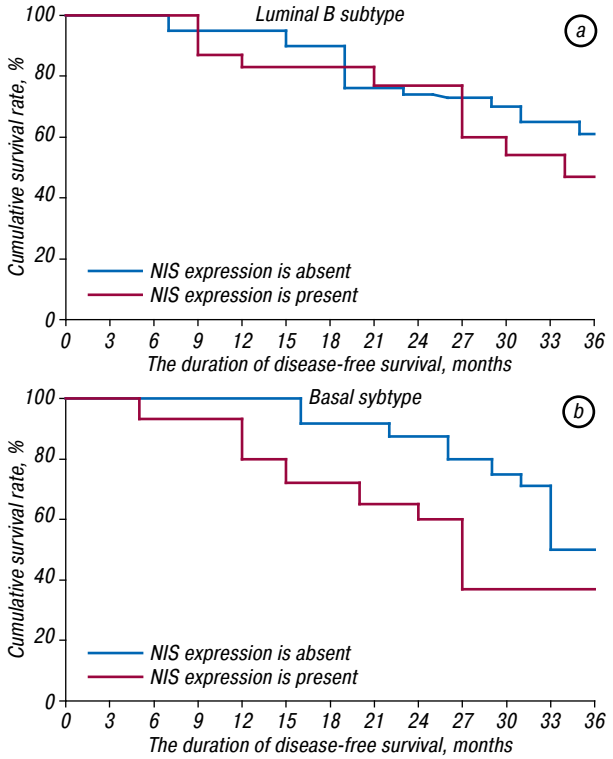

Fig. 4 Disease-free survival (Kaplan — Meier) of patients with BC of luminal B (*a*) and basal (*b*) subtypes depending on NIS expression in tumor tissue (long-rank test, $p < 0.05$)

The results indicate that NIS can be used as an objective criterion for determining the sensitivity of luminal B and basal BC subtypes to NACT, which will provide improved treatment outcomes in this group of patients.

REFERENCES

1. Cancer in Ukraine, 2014–2015. Bul Nat Cancer Register of Ukraine 2016; (17): 46–7 (in Ukrainian).

2. **Hennigs A, Riedel F, Gondos A.** Prognosis of breast cancer molecular subtypes in routine clinical care: A large prospective cohort study. BMC Cancer 2016; **16**: 734.

3. **Langlands FE, Horgan K, Dodwell DD, *et al.*** Breast cancer subtypes: response to radiotherapy and potential radiosensitisation. Br J Radiol 2013; **86**: 623–37.

4. **Yersal O, Barutca S.** Biological subtypes of breast cancer: Prognostic and therapeutic implications. World J Clin Oncol 2014; **5**: 412–24.

5. **Dai X, Li T, Bai Z, Yang Y, *et al.*** Breast cancer intrinsic subtype classification, clinical use and future trends. Am J Cancer Res 2015; **5**: 2929–43.

6. **Yardley DA.** Drug resistance and the role of combination chemotherapy in improving patient outcomes. Int J Breast Cancer 2013; doi: 10.1155/2013/137414.

7. **O'Reilly EA, Gubbins L, Sharma S, *et al.*** The fate of chemoresistance in triple negative breast cancer (TNBC). BBA Clin 2015; **12**: 257–75.

8. **Semba RD, Delange F.** Iodine in human milk: perspectives for infant health. Nutr Rev 2001; **59**: 269–78.

9. **Smyth PP, Shering SG, Kilban MT, *et al.*** Serum thyroid peroxidase autoantibodies, thyroid volume, and outcome in breast carcinoma. J Clin Endocrin Metab 1998; **83**: 2711–6.

10. **Renier C, Yao C, Goris M, *et al.*** Endogenous NIS expression in triple-negative breast cancers. Ann Surg Oncol 2009; **16**: 962–8.

11. **Hansen RK, Bissell MJ.** Tissue architecture and breast cancer: the role of extracellular matrix and steroid hormones MicroRNA signatures: clinical biomarkers for the diagnosis and treatment of breast cancer. Endocr Relat Cancer 2000; **17**: 95–113.

12. **Ryan J, Curran C, Hennessy E, *et al.*** The sodium iodide-symporter (NIS) and potential regulators in normal, benign and malignant human breast tissue. PLoS One 2011; **6**: 160–3.

13. **Lukianova NY, Andriiv AV, Chekhun VF.** Correlation of iodine symporter expression in highly and low malignant cell lines of human breast cancer differed in their sensitivity to doxorubicin. Exp Oncol 2016; **38**; 169–71.

14. **Agrawal A, Purandare N, Shah S, *et al.*** Response assessment in metronomic chemotherapy: RECIST or PERCIST? Indian J Nucl Med 2014; **29**: 74–80.

15. **Smolanka II, Sklar SY, Ivankov OM, *et al.*** The effectiveness of neoadjuvant chemotherapy in patients with breast cancer. Medical pathomorphosis. Clin Oncol 2013; **2**: 1–6 (in Russian).

16. **McClelland RA, Wilson D, Leake R, *et al.*** A multicentre study into the reliability of steroid receptor immunocytochemical assay quantification. Eur J Cancer 1991; **27**: 711–5.