TEICHOIC ACID DIFFERENTIALLY MODULATES TLR4 EXPRESSION IN SENSITIVE AND RESISTANT TO CISPLATIN SMALL CELL LUNG CARCINOMA H69 CELLS

The Toll-like receptor family plays crucial role in the innate immune system, recognizing the molecular structures associated with pathogens derived from different microbes. TLRs also recognize the molecular structures associated with damage associated molecular patterns (DAMPs) and DAMPs associated with cell death. There is evidence that TLRs can contribute to the development of cancer, as well as to the response of cancer cells to therapeutic interventions. TLRs are involved in the development of cancer, as well as to the response of cancer cells to therapeutic interventions.

Introduction. The Toll-like receptor family (TLR) is a member of the interleukin-1 (IL-1R) / TLR receptor superfamily. This superfamly was described in 1998 as a family of type I transmembrane proteins containing an intracellular TIR domain with common basic structure [1]. TLRs play crucial role in the innate immune system, recognizing the...
molecular structures associated with pathogens derived from different microbes. TLRs also recognize the molecular structures associated with damage associated with certain diseases, such as cancer [2].

TLR activation was associated with both tumor suppression and tumor progression. Tumors are infiltrated by different types of immune cells, and immune cells can be the main cell population in the tumor microenvironment. Therefore, it is increasingly recognized that inflammatory processes play key role in tumorigenesis [3].

TLR ligands are often used as adjuncts to enhance immunogenicity of vaccines in anticancer therapy [4]. Such ligands are the cell wall biopolymers derived from gram-positive microorganisms Staphylococcus aureus, the teichoic acids (TA). Our previous studies have shown that TA in combination with the bimetallic copper and cadmium complex with ethylenediamine (PO244) exacerbated the antitumor activity of the latter. However, incubation of primary Lewis lung carcinoma cells with TA leads to an increase in aneuploidy cell population and a decrease in apoptotic cell levels. But in combination with PO244, TA provided 2-fold increase in the level of LLC apoptotic cells and reduced the population of LLC cells in the proliferative pool (G2 / M + S phase) to 40%, compared to 65% in control [5]. TLR4 expression is characteristic of innate immunity. However, the activation of TLR4 in the tumor process may be associated with tumor initiation and progression. Different types of tumors may have different patterns of TLR4 involvement during tumorigenesis or tumor progression [6]. In addition, in some tumor models, TLR2 and TLR4 polymorphisms are known to affect cancer risk, which means that a genetic difference in specific TLR may be associated with specific tumor behavior [7]. Cisplatin therapy is widely used anticancer treatment for various neoplasms. However, this compound causes side effects in healthy tissues and body systems, and causes drug resistance [8,9]. There is various evidence regarding the involvement of TLRs, in particular TLR4, in the emergence of cisplatin resistance.

Therefore, the purpose of our study was to determine the expression level of TLR4 in cisplatin-sensitive and cisplatin-resistant lung cancer cells.

Materials and method. Cells line NCI-H69/CPR (human small cell lung carcinoma) is a drug resistant subline of NCI-H69 (Sigma Catalogue number. 91091802) were used to determine the expression level of TLR4. Drug resistance was developed by addition of cisplatin in a stepwise increment to the growth medium of the parental line. The cell line exhibits a 5-fold resistance to cisplatin and is cross resistant to melphalan. The cells were incubated in culture medium RPMI 1640+2mM glutamine + 0.4 μg/ml cisplatin + 10% fetal bovine serum (FBS). Incubation of cells with teichoic acid (1 μg/ml) derived from gram-positive microorganisms Staphylococcus aureus was performed under standard conditions for two days.

Total RNA was isolated by phenol–chloroform extraction and the "Ribo-zol" kit ("AmpliSens"). RNA concentration in all samples was measured by Thermo Scientific Nano Drop-1000 (Thermo Fisher Scientific, USA) and samples were diluted to 200 ng/μl. cDNA was obtained from total RNA by RT-PCR using "High Capacity cDNA Reverse Transcription Kit" (Applied Biosystems, USA). The reverse transcription reaction was run under the following conditions: 25 °C – 10 min, 37 °C – 120 min and 85 °C – 5 sec. cDNA was diluted in in half with DNA buffer. TLR4 expression level was evaluated by real-time PCR on 7500 Real-Time PCR Systems ("Applied Biosystems", USA) using specific primers and fluorochromeSYBRGreen ("Applied Biosystems", USA). GAPDH was used to normalize levels of mRNA for the relative quantification method of analysis. TLR4 sequence (f- CTGTGTCACTCAGGGAGCC, r- GCAGGTAGGGGAGAACCC) and GAPDH sequence (f- GCCAAGGTCACTCCAGGAAAACCC, r- GCCGCTTCACCCACCTTTGAC) were constructed by Primer Express® Software v3.0 (Applied Biosystems, USA). 45 cycles real-time PCR (94 °C – 15 sec, 65 °C – 15 sec and 72 °C – 30 sec) were run on 7300/7500 Real-Time PCR Systems, "Applied Biosystems", USA. Calculations were performed using the ΔΔCt relative quantification method.

Results. Lung cancer cells sensitive (NCI-H69) and resistant to cisplatin (NCI-H69/CPR) differ in a number of morph functional characteristics, especially proliferative and adhesive properties (Fig. 1).

Incubation of NCI-H69 and NCI-H69/CPR cells was carried out for one and two days without and with TA at a concentration of 1 μg / ml. Through after day of cultivation, it was found that in the wild type cells, the expression level of TLR4 was 0.00261±0.000432 a.u., whereas on the second day of incubation this indicator increased slightly

Fig. 1. Morphological features of WT (NCI-H69) and resistant to cisplatin (NCI-H69/CPR) small cell lung carcinoma NCI-H69 cells. Cells were imaged under phase-contrast microscopy.
and was 0.002814±0.000202 a.u. With respect to the cisplatin-resistant cell line, almost twice the expression level of TLR4 was detected compared to the WT cells and this indicator did not change on the second day of incubation of cells under standard conditions (Fig. 2).

![Fig. 2. TLR4 expression level in NCI-H69 and NCI-H69 / CPR cells under standard incubation conditions](image)

*-*P<0.05 vs control (WT NCI-H69 cells)

Since no differences in the expression of TLRs at 1 and 2 days of incubation were detected, the expression of TLR4 under the action of TA was determined after 2 days of incubation. Preincubation of cells of both lines for two days with TA led to the following results: in WT cells it was possible to observe the increase of expression of TLR4 gene, whereas in the cisplatin-resistant cells we revealed a pronounced inhibitory effect of the TLR ligand (Fig. 3).

![Fig. 3. TLR4 expression level in NCI-H69 and NCI-H69 / CPR cells by adding TA](image)

*-*P<0.05 vs WT NCI-H69, ^-*P<0.05 vs cells without TA

Thus, it was shown that the TLR 4 expression level was almost twice lower in the WT cells than in the cisplatin-resistant cells. The effect of teichoic acid resulted in an increase of TLR4 expression in the WT cells line strain 1.3-times compared to control, whereas in cisplatin-resistant cells TLR4 expression level decreased 4-times compared to the sample without the effect of teichoic acid. The research conducted by Lewison carcinogenic lungs indicates a significant role of TLR4 not only in tumor growth but also in migration [10]. The refore, inhibition of expression of the receptors may be considered as a new strategy for antitumor and anticlastic action. It is also possible to use inhibition of the expression of TLR by specific ligands in combination with antitumor agents in resistant tumors.

**Conclusion.** Thus, in cisplatin-resistant lung cancer cells found high expression of TLR4 can be inhibited by adding a ligand to TLRs.
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ТЕЙХОЕВАЯ КИСЛОТА МОДУЛИРУЕТ ЭКСПРЕССИЮ ТОЛЛ-ПОДОБНЫХ РЕЦЕПТОРОВ 4 ЧУВСТВИТЕЛЬНЫХ И РЕЗИСТЕНТНЫХ К ДЕЙСТВИЮ ЦИСПЛАТИНА ЛИНИЙ КЛЕТОК МЕЛКОКЛЕТОЧНОГО РАКА ЛЕГКОГО

Семейство Толл-подобных рецепторов (ТПР) играет важную роль в механизмах развития врожденного иммунного ответа, распознавая молекулярные структуры патогенных микроорганизмов. ТПР способны также влиять на развитие противоопухолевого иммунного ответа. Они могут как стимулировать опухолевый рост, так и подавлять его. Стимулирование роста опухолевых клеток может быть связано с приобретением лекарственной резистентности. Данные экспрессии ТПР4 при опухолевом росте противоречивы. Именно поэтому целью исследования являлось определение уровня экспрессии ТПР4 в клетках рака легкого, чувствительных и резистентных к цисплатину. Клетки линий NCI-H69 и NCI-H69 / CPR (мелкоклеточный рак легкого человека) использовали для определения уровня экспрессии ТПР4. Инкубацию клеток с тейхоевой кислотой (1 мкг/мл) проводили в стандартных условиях в течение двух суток. Уровень экспрессии TLR4 в клетках определяли с помощью RT-PCR при 7500 ПЦР-системах в реальном времени ("Applied Bio systems", США), специфических праймеров и асимметричным циано-катионным фторхоромере SYBR Green (C32H37N4S +) при Амакс = 488 нм флуоресценции при Амакс = 522 нм. Было показано, что уровень экспрессии ТПР4 был почти в два раза ниже в клетках WT H69 по сравнению с клетками H69, резистентных к цисплатину. В отличии от этого, показано, что тейхоевая кислота имела противоположное влияние на уровень экспрессии ТПР4. В клетках WT H69 уровень экспрессии возрос в 1,3 раза, в клетках H69, резистентных к цисплатину — снизился в 4 раза по сравнению с контрольными образцами без добавления тейхоевой кислоты. Итак, в клетках рака легкого с высокой экспрессией ТПР4, резистентных к цисплатину, можно ингибировать экспрессию, добавляя лиганд к ТПР.

Ключевые слова: экспрессия ТПР4, линия клеток, резистентность к цисплатину NCI-H69, тейхоевая кислота.