Abstract

Introduction: Genome-wide analysis identified a new class of non-coding RNAs, long non-coding RNAs (lncRNAs) – longer than 200 bp, which play an important role in many biological processes. Disturbances in their expression can lead to many diseases, including cancer. There are reports considering long non-coding RNAs as potential biomarkers for the diagnosis and clinical prognosis of cancer. This may be because lncRNAs regulate the level of specific proteins involved in the tumorigenesis, directly or indirectly (by inhibiting/ activating a protein involved in the signaling pathway, e.g. β-catenin) – because they occur in the cell nucleus and in the cytoplasm. Additionally, lncRNAs are tissue-specific and their expression can be regulated by the cell depending on its condition.

Lung cancer is the leading cause of cancer death worldwide, accounting for approximately 25% of all cancer cases worldwide. In addition, 80% of lung cancer patients are classified as non-small cell lung cancer (NSCLC) and 20% as small cell lung cancer (SCLC).

Understanding the processes regulated by long non-coding RNAs is crucial not only from a cognitive point of view but also for the development of new biomarkers and effective therapeutic targets in cancer patients.

The aim of the project was to evaluate the expression level of lncCDH5-3:3 and lncCXCR4-6:1 in non-small cell lung cancer cells and determine their role in the invasiveness of cancer cells.

Materials and methods: The biological material used in this study was tumors collected from 20 patients from which RNA was isolated. Then, the expression of lncCDH5-3:3 and lncCXCR4-6:1 was assessed and correlated with the stage of disease and the histopathological type.

Next, *in-silico* analyses were performed to determine the stability of the tested lncRNAs, and in the case of lncCXCR4-6:1 the probable protein binding site. In the next step, knockout was performed using the px459V2.0 plasmid and the design, specific for lncCDH5-3:3 and lncCXCR4-6:1 gRNAs. Parallelly, overexpression of mentioned lncRNA`s was performed using the pcDNA3.1. Deletions were made in the lncCXCR4-6:1 sequence with a length of 440bp, 493bp, and 540bp. As mentioned above, genetic engineering methods were used in lung cancer cell lines: H1703 (squamous cell carcinoma), H1975, and A549 (lung adenocarcinoma cell lines).

In the next step, the mRNA level of EpCAM, E-cadherin, Oct-4 (POU5F1), and CXCR-4 in cells after KO lncCDH5-3:3 and in NSCLC patients was evaluated. Subsequently, the percentage of proliferating and apoptotic cells, percentage of cells in the SubG1 phase (only for studies related to IncCDH5-3: 3), G0 / G1, S, and G2 / M was determined. These assays were performed in KO cells and in cells that overexpressed lncCDH5-3:3 and lncCXCR4-6:1. In addition, these tests were also performed after deletions which were performed in the lncCXCR4-6:1 sequence. Additionally, to confirm the influence of lncCDH5-3: 3 on the apoptotic process, the percentage of live, apoptotic and dead cells were determined, based on the activity of pro-apoptotic caspases 3 and 7. On the other hand, in the case of lncCXCR4-6:1, the activity of the EGFR/ ERK1/2 pathway was determined as well as the level of the transcription factor Nf-κβ and its activity.

The last step was to determine the level of EpCAM, E-cadherin, EV-cadherin (in the case of lncCDH5-3:3), CXCR-4 (in the case of lncCXCR4-6:1), Oct-4 (POU5F1), phosphorylated β-catenin, the total concentration of Akt and phosphorylated Akt, the total concentration of

ERK1/2 and phosphorylated ERK1/2 during overexpression of the lncRNAs in as well as after their KO and deletions within the lncCXCR4-6:1 sequence. Additionally, to determine the invasive capabilities of the cancer cells after KO or overexpression of lncCXCR4-6:1, a scratch test was performed and the cells were monitored until the scratch was fully overgrown.

Conclusions: The results presented in this study prove that:

1. lncCDH5-3:3 regulates apoptosis by activating pro-apoptotic caspases 3 and 7,
2. lncCDH5-3:3 regulates proliferation by increasing the expression of EpCAM, Oct-4 and Nanog and the activity of Akt, ERK1/2,
3. lncCDH5-3:3 regulates the invasive capabilities of cancer cells by activating the expression of E-cadherin and EV-cadherin,
4. a cancer cell can control lncCDH5-3:3 expression level by activating proteins involved in proliferation or migration.
5. lncCXCR4-6:1 regulates apoptosis and proliferation of cancer cells by regulating the expression of transcription factors Oct-4, Nanog, as well as proteins: Akt, ERK1/2, and CXCR-4 and phosphorylation of the receptor for EGF, Akt, ERK1/2, and β-catenin,
6. lncCXCR4-6:1 regulates the signaling pathways: EGFR/Akt/ERK1/2, CXCR-4/Akt/NF-κβ, and β-catenin/EpCAM/Oct-4/Nanog/E-cadherin,
7. Deletion of fragments within the sequence of lncCXCR4-6:1 proved that mentioned lncRNA regulates the expression level of CXCR-4,
8. lncCXCR4-6: 1 regulates NF-κβ activity only in squamous cell carcinoma cells.
9. the L858R mutation within EGFR triggers compensatory effects which should increase proliferation and stopping apoptosis.