Transient multiple transfection of miR-181a into MCF-7 breast cancer cells induces irreversible resistance to tamoxifen

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INTRODUCTION

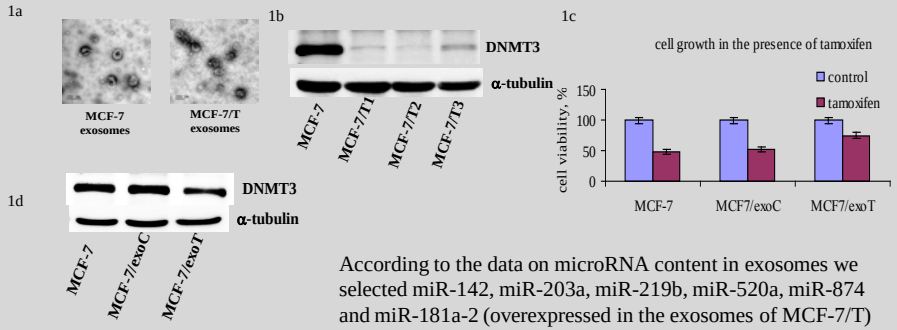
Breast cancer (BC) resistance to antiestrogens is one of the main problems that limit the efficacy of chemotherapy. Exosomes, microvesicles secreted and absorbed by cells, play an important role in the development and transmission of resistance of tumor cells. Exosomes are enriched with microRNAs taking part in the regulation of target genes. The aim of this work was to identify the microRNAs involved in the development of resistance and to study the effects of their transfection into BC cells.

MATERIALS AND METHODS

MCF-7/T subline was obtained via prolonged cultivation of MCF-7 parent cells in the presence of tamoxifen. Exosomes were isolated by ultracentrifugation. MicroRNA content was studied by NGS. Transient multiple transfections of microRNAs were performed in the cells. Cell growth rates were measured by MTT. Protein levels were evaluated by immunoblotting. Methylation levels were accessed by bisulfite sequencing.

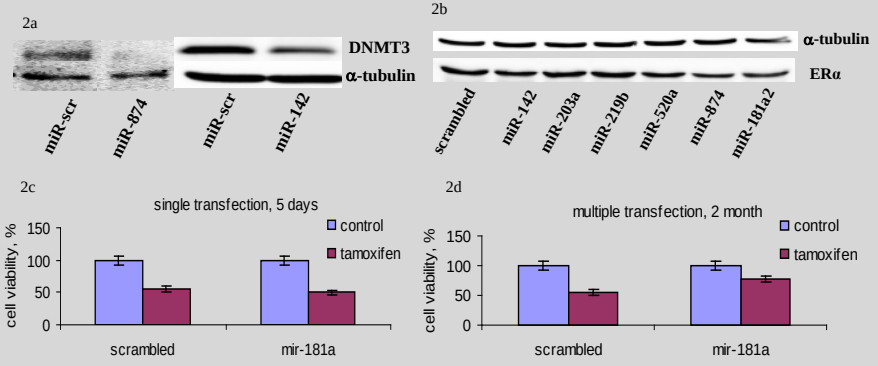
RESULTS

Exosomes of resistant cells can transmit tamoxifen resistance. Exosomes isolated from MCF-7 parent cells and MCF-7/T resistant subline are depicted in (1a). We have found that in 3 tamoxifen-resistant sublines obtained independently DNMT3 level is being suppressed (1b). Cells incubated with exosomes by MCF-7/T became tamoxifen-resistant (1c) and shown decreased DNMT3 level (1d).



According to the data on microRNA content in exosomes we selected miR-142, miR-203a, miR-219b, miR-520a, miR-874 and miR-181a-2 (overexpressed in the exosomes of MCF-7/T) to study their effects.

Some of these microRNA (miR-142, miR-874, miR-181a-2) transfected into MCF-7 decreased DMNT3 level (2a, 3b). MiR-181a-2 effectively suppressed ERα level (2b), so miR-181a-2 was chosen for further experiments. Not single but multiple (over 20) transfections of miR-181a-2 even in 2 month after the last one make cells resistant to tamoxifen (2c, 2d).

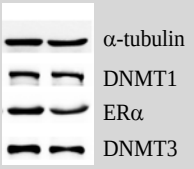


MiR-181a-2 has 2 binding sites in 3`UTR of DNMT3A and 2 sites in ESR1 according to TargetScan (3a). In the cells after multiple miR-181a-2 transfections 2 month prior to experiment DNMT3A level and ERα level remain decreased (3b)

3a

	Predicted consequential pairing of target region (top) and miRNA (bottom)	Site type
Position 2974-2981 of DNMT3A 3' UTR	5' ...GGAGCCUCUGCCCCCUCAGUGGA...	8mer
hsa-miR-181a-2-3p	3' CCAUGUCAGUUGCCAGUCACCA	
Position 3716-3722 of DNMT3A 3' UTR	5' ...GGCAGAGGUUAGAGGCAGUGGAG...	7mer-A1
hsa-miR-181a-2-3p	3' CCAUGUCAGUUGCCAGUCACCA	
	Predicted consequential pairing of target region (top) and miRNA (bottom)	**Site type**
Position 2354-2360 of ESR1 3' UTR	5' ...ACAGUAGCUAAUGGGUCAGUGGG...	7mer-m8
hsa-miR-181a-2-3p	3' CCAUGUCAGUUGCCAGUCACCA	
Position 2470-2476 of ESR1 3' UTR	5' ...AGCCAAACAAUUAUACAGUGGAA...	7mer-A1
hsa-miR-181a-2-3p	3' CCAUGUCAGUUGCCAGUCACCA	

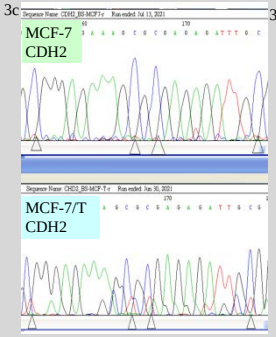
3b



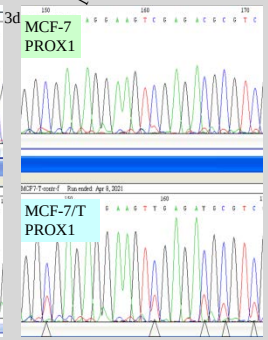
DNMT3A is required for genome-wide *de novo* methylation and is essential for the establishment of DNA methylation patterns during development. Bisulfite sequencing was used to study the differences in the methylation levels in MCF-7/T compared to MCF-7.

There was no difference in DNMT3 methylation level (data not shown), but **CDH2** (3c) and **PROX1** (3d) genes **in MCF-7/T were demethylated**. These genes can be considered as oncogenes and can be reexpressed in resistant cells due to DNMT3A loss.

3c



3d



CONCLUSIONS

The transient multiple transfection of miR-181a-2 into MCF-7 cells induces the irreversible tamoxifen resistance demonstrating the important role of this microRNA in the formation of the resistant phenotype

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