Preclinical data with siRNA targeting the Wnt pathway for breast cancer treatment

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Introduction

The WNT family of secreted-type glycoproteins play key roles in carcinogenesis and embryogenesis. Signals of glycoprotein WNTs are transduced through seven-transmembrane-type WNT receptors encoded by Frizzled (FZD) genes to the ß-catenin - TCF pathway, the c-Jun-N-terminal kinase (JNK) pathway or the Ca^{2+}-releasing pathway. In human breast cancer, evidence of β-catenin accumulation implies that the canonical Wnt signaling pathway is active in over 50% of carcinomas.

Aim:

The aim of present study was focused on the effect of the *Wnt pathway* silencing in triggering of apoptosis in breast cancer MCF-7 cells. Flow cytometry, light-, confocal microscopy and viability/cytotoxicity tests were used for evaluation of the Wnt1 protein level, percentage of apoptotic cells and morphological features of cell death.

Materials and methods:

Breast cancer MCF-7 cells were transfected with fifteen siRNAs sequences (W1-W15) specific to the target gene designed in Celon Pharma and one from literature data (WP) in concentration 50nM for 24-48h using Lipofectamine RNAi MAX (Invitrogen). The sequence with the best efficiency in silencing was used for further experiments. MCF-7 cells were transfected with 40nM of W15 siRNA for 48h (two doses every 24 hours). Effect of a silencing was observed using light and confocal microscopy and measured by flow cytometry, western blotting and luminescence. The results were statistically evaluated by ANOVA and Tukey's multiple range tests using Graph Pad Prism® 4.0. $P \leq 0.05$ was regarded as significant, at $P \leq 0.01$ as highly significant and $p \leq 0{,}001$ as very high significant.

Results

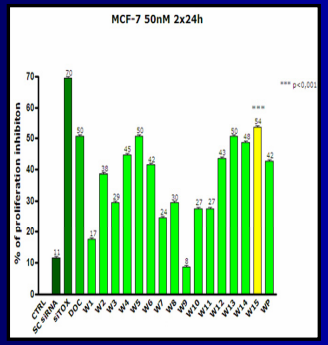


Fig. 1 Percent of proliferation inhibition after transfection of MCF-7 cells with fifteen siRNAs sequences specific to the targeted gene in concentration 50nM for 48h. Cells viability was measured using MTS test (Promega).

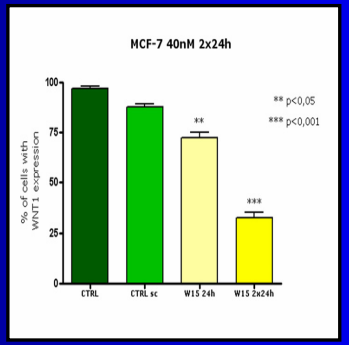


Fig. 2 Percent of MCF-7 cells showing expression of the gene after transfection with 40nM of W15 siRNA (one dose for 24h) and two doses every 24h analysed using flow cytometry.

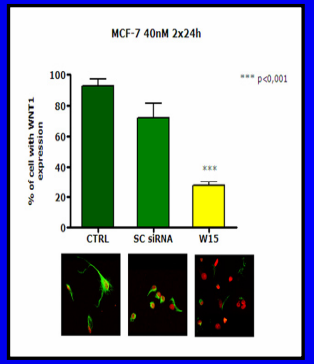


Fig. 3 Flow cytometry and confocal microscopy analysis of the target gene expression after transfection with 40nM W15 siRNA for 48h (two doses every 24 hours).

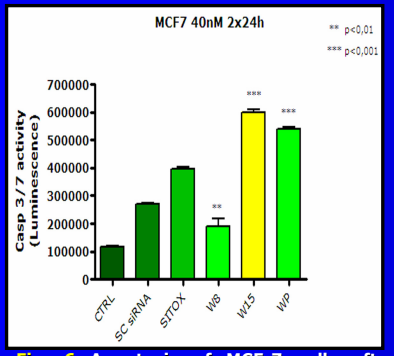
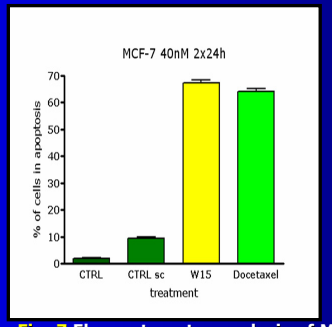


Fig. 6 Apoptosis of MCF-7 cells after transfection with 40nM W15 siRNA for 48h (two doses every 24 hours) measured by the activation of caspase 3/7 (luminescence analysis).



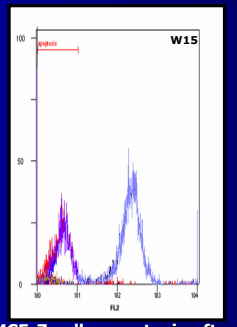


Fig. 7 Flow cytometry analysis of MCF-7 cells apoptosis after 40nM W15 siRNA transfection (two doses every 24h) 15nM of docetaxel was used as a positive control of apoptosis induction in MCF-7 cells

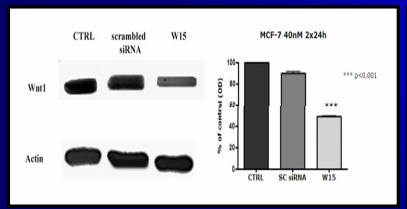


Fig. 4 Effect of knock down of *the target* gene on protein level analyzed by Western blot after transfection with 40nM W15 siRNA for 48h (two doses every 24 hours).

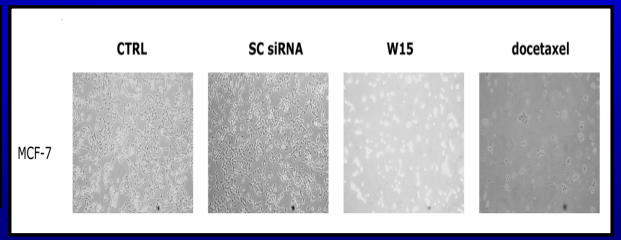


Fig. 5 Morphological changes typical to apoptosis of MCF-7 cells after transfection with 40nM W15 siRNA for 48h (two doses every 24 hours).

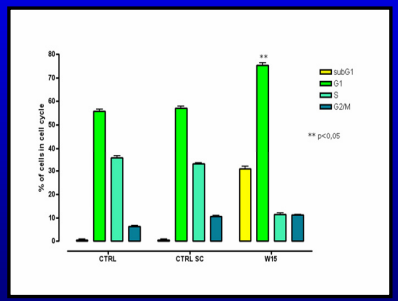


Fig. 8 Blocking of cell cycle in G1 phase after transfection of MCF-7 cells with 40nM W15 siRNA (two doses every 24h) .

Conclusions:

- Transfection of MCF-7 cells with siRNA specific to the gene from Wnt pathway very significantly decrease Wnt1 protein expression within 48h after 40nM dose of siRNA
- Reduction of the protein expression is significantly corelated with induction of MCF-7 cells apoptosis and cell cycle blocking in G1 phase
- Silencing of the gene in breast cancer cells induced apoptosis with the same efficiency as commonly used anticancer drug (docetaxel)
- This results show that siRNA specific to the gene from Wnt pathway can be a useful tool for breast cancer therapy