**Introduction**

While prevailed clinical use GnRH agonist, the underlying mechanism surrounding signaling through the GnRH receptor is not yet clearly understood, precluding classical research methods. We propose to clarify the effect of GnRH agonist on proliferation of hormone refractory prostate cancer cell lines with transferable RNA interference, i.e. short hairpin RNA (shRNA), for the type 1 GnRH receptor (GnRHR I).
Using the androgen-independent prostate cancer cell line DU 145, cellular proliferation under graded doses (10^{-9}, 10^{-7}, and control) of GnRH agonist (Leuprolide) were measured using the XTT colorimetric assay, before and after knock down of GnRHR I, by transfection of sh-GnRHR I- pSUPER.

RT-PCR and Western blot analysis were used for measurement of the degree of silencing of GNRHR I.
The difference in cellular proliferation pattern of DU145 and LNCap-FGC cell lines, under same dosage of leuprolide. While LNCap-FGC cells showed no inhibition, DU145 cells showed significant inhibition when leuprolide was applied, compared to control group. (*; p <0.05, compared to control group)
Real-time PCR of transfected shRNA showed significant decrease of type 1 GnRH receptor gene expression at 48 hours. (*; p < 0.05, compared to control)

Western blot analysis demonstrated effective reduction in GnRH receptor type 1 expression.
The effect of silencing GnRHR1 on cellular proliferation pattern. When cultured during a period of 72 hours, the pattern of DU145 cells showed time-dependent significant increase (*; p=0.007, compared to 24 hours, †; p=0.005, compared to 48 hours).

After silencing of GnRHR1, similar pattern of time-dependent growth had been revealed (*; p=0.03, †; p=0.001).
Results

Comparison of cellular proliferation pattern before and after silencing of GnRHR1. After transfection of shRNA, antiproliferative effect of leuprolide was disappreaed. Rather in highest dosate, leuprolide promoted proliferation of cells, compared with control group. (*; p <0.05, compared to control group)
Conclusion

These data obviously illustrated that the antiproliferative effect of leuprolide, GnRH agonist, is mediated by GnRH type 1 receptor.