The relation between TORC1 and TORC2 expression and response on mTOR inhibitors treatment in uterine sarcoma and carcinosarcoma cell lines.

Marcin Bobiński[1,2], Karolina Okła[1,2], Wiesława Bednarek[2], Iwona Wertel[2], Anna Wawruszak[3], Jan Kotarski[1,2]

1. European Network for Individualised Treatment of Endometrial Cancer
2. I st Department of Gynaecological Oncology and Gynaecology, Medical University of Lublin, Poland
3. Department of Biochemistry and Molecular Biology, Medical University of Lublin, Poland

Introduction

mTOR inhibitors (mTORi) is one of the most promising group of anticancer agents considered to be active against uterine mesenchymal malignancies. A few recently published trials revealed their activity but in most of them only a part of patients responded to the treatment. No biomarkers allowing to assess the response to mTOR inhibitors were identified so far. Taking into consideration the mechanisms of intracellular activity of first (rapamycin) and second (INK 128) generation of mTORi, targeting TORC1 and both TORC1 and TORC2, respectively, we aimed to assess the expression of mentioned mTOR complexes in selected uterine sarcoma and carcinosarcoma cell lines and to analyze their relation to the response to rapamycin and INK 128 treatment.

Materials and methods

Four cell lines derived from uterine leiomyosarcoma (MES-SA), endometrial stromal sarcoma (ESS-1), carcinosarcoma (SKUT-1, SKUT1B) were selected to be used in the study. The control normal line was HSF (human skin fibroblasts). Cell from cultures were harvested and used to prepare paraffin embaded slides. The expression of TORC1 and TORC 2 was visualized using immunochemistry according to antibody manufacturer recommendations and assessed by semiquantitive method as no expression/weak expression/strong expression/very strong expression.

To reveal the response to mTORi treatment, cells were cultured in presence of rapamycin in concentrations 0.25–2500 ng/ml and INK128 in concentrations 0.1–100 ng/ml for 96 h. Cell viability was assessed using MTT test. The response of cell lines was expressed as IC50 value.

Results

Very strong expression of TORC1 was detected in MES-SA cell line, weak In SKUT-1 and SKUT-1B, no expression was found in HSF an ESS-1 lines. The expression of TORC2 was considered as very strong in MES-SA cell line, as weak in HSF, SKUT1, SKUT-1, and ESS-1.

IC50 for rapamycin was achievable for ESS-1 and MES-SA and valued 661.6 ng/ml and 1.1 ng/ml respectively. For INK 128 it was measured for SKUT-1, SKUT1B, ESS-1 and MES-SA the values were figured as 214.2 ng/ml, 78.8 ng/ml, 18.7 ng/ml and 3.7 ng/ml respectively.

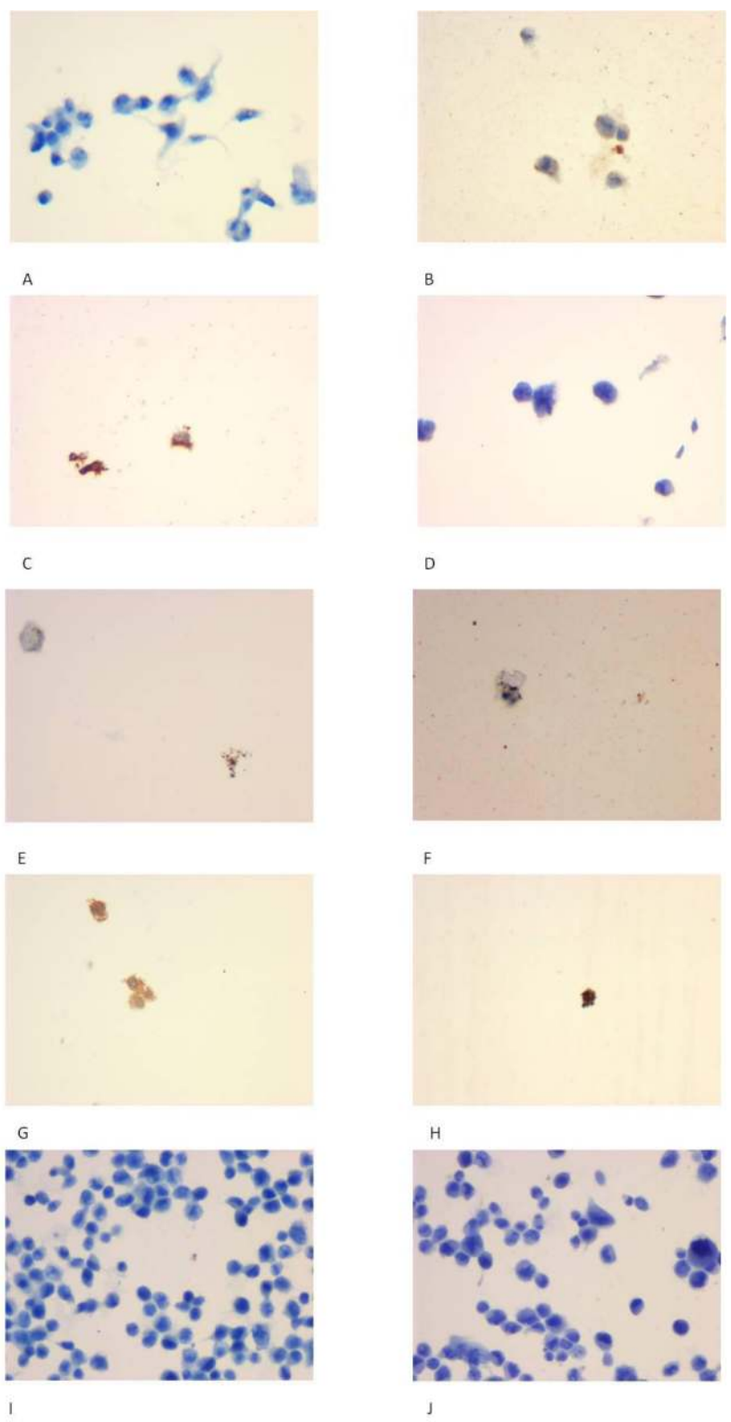

Fig.1. Immunochemical reaction with anti-TORC1 (A, C, E, G and I) and anti-TORC2 (B, D, F, H and J) antibodies in HSF, SKUT-1, SKUT-1B, MES-SA, ESS-1 cell lines, respectively.

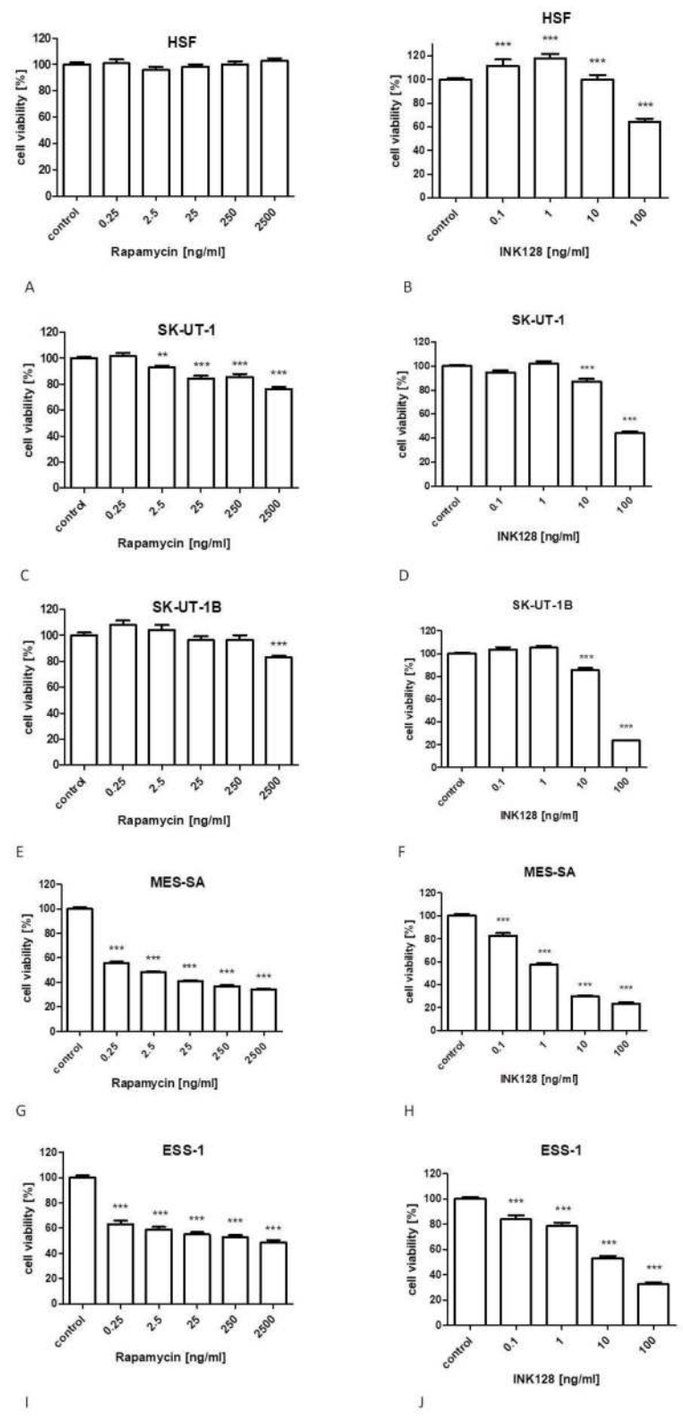

Fig.2. The influence of rapamycin (A, C, E, G and I) and INK0128 (MLN0128) (B, D, F, H and J) on the proliferation of HSF, SKUT-1, SKUT-1B, MES-SA, ESS-1 cell lines, respectively. (*p<0.05, **p<0.01, ***p<0.001 were considered as statistically significant).

Conclusion

Our data show that very strong expression of TORC complexes is related to good response to mTORi as it was observed in leiomyosarcoma cell line. We noted that endometrial stromal sarcoma cell line presented some sensitivity for rapamycin in spite of no expression of TROC1. This finding can be explained by limited sensitivity of immunochemistry, and it is worth to point out that IC50 value was over 600 times higher comparing to leiomyosarcoma cell line response. Weak expression of both TORC complexes in carcinosarcoma cell lines corresponds with relatively high concentration of INK 128 necessary to achieve half maximal inhibition of cells viability. Interestingly, we did not observe significant impact of tested substances on normal cells (human skin fibroblasts) in concentrations that were sufficient to affect the most sensitive cancer cell lines.