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In vitro characterization of an anti-HER2 affibody-monomethyl auristatin E conjugate, in HER2-positive breast cancer cells

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Introduction

The human epidermal growth factor receptor 2 (HER2) is a tyrosine kinase receptor which belongs to the family of the epidermal growth factor receptors (EGFRs). Amplification of HER2 gene is observed in 20–30% of human cancers, especially breast and ovarian cancers[1], and its overexpression is correlated with poor prognosis and worse clinical outcomes [2].
Antibody-Drug Conjugates (ADCs) represent a successful class of anticancer agents that combine the selectivity of mAbs with the cytotoxic potency of chemotherapeutic agent [19]. One of the major limitations of these molecules is their large size (150 kDa) that limits their ability to penetrate solid tumors [3]. In addition, due to their long serum half-life and slow blood clearance, they are not suitable for radioimmunotherapy or imaging purposes [3]. A promising approach for the development of high-affinity tumor targeting ADCs is the use of engineered protein drugs, such as affibody molecules that represent a valuable alternative to monoclonal antibodies (mAbs) in cancer-targeted therapy.

Aims

In the present work, we tested the *in vitro* efficacy of $Z_{HER2:2891}$DCS affibody conjugated with the cytotoxic antimitotic agent auristatin E (MMAE) on several parameters such as cell viability, proliferation and apoptosis. The effects of $Z_{HER2:2891}$DCS-MMAE were compared with the clinically approved monoclonal antibody trastuzumab (Herceptin®) and $Z_{HER2:2891}$DCS not conjugated with MMAE, used as negative control. To demonstrate that $Z_{HER2:2891}$DCS-MMAE can selectively target HER2 overexpressing tumor cells we used two different cell lines: the human adenocarcinoma cell line SK-BR-3 that over-expresses HER2 and the triple-negative breast cancer cell line MDA-MB-231.

Methods

Cell culture:
- Human breast cancers SK-BR-3 (high HER-expressing cells)
- Human breast cancers MDA-MB-231 (basal HER-expressing cells)

Cell viability by Formazan salts:
SK-BR-3 and MDA-MB-231 were seeded in 24 well-plates at density of $1x10^5$ cells/well and $7x10^4$ cells/well, respectively, and then let grow for 24 hours at 37°C. Cell viability upon treatments was evaluated by the MTT method

Cell proliferation assay:
SK-BR-3 and MDA-MB-231 were seeded in 24-well plates at density of $2x10^6$ cells/well. After 24 hours, media were removed, and cells were incubated for 72 hours with medium containing 0.4% FBS to synchronize cells at G_0 phase of the cell cycle. After 72 hours, control dishes were counted with the Coulter Counter (Beckman Coulter, Life Scientific, Milan, Italy) and this was considered the "basal" number of cells at T0. Consequently, cells were treated with drugs for 24, 48 and 96 hours. Cells number were measured and compared to zero time-point.

Apoptosis analysis:
Cell apoptosis was performed using Annexin V-FITC Apoptosis Detection kit (Sigma-Aldrich) according to manufacturer instructions. The percentage of apoptotic cells was evaluated with a flow cytometer (ACEA Biosciences NovoCyte, San Diego, CA)

Conclusion

In conclusion, our experimental data demonstrate that the cytotoxic conjugate formed by the anti HER2 affibody and monomethyl auristatin E efficiently interacts with HER2-expressing cancer cells *in vitro* allowing for a selective and specific delivery of the cytotoxic payload. This demonstrates that affibody may be used to target HER2 expressing cells. This technique might allow to avoid some of the problems encountered by using trastuzumab in clinics, such as poor tissue penetration due to its high molecular weight.

Results

Fig.1

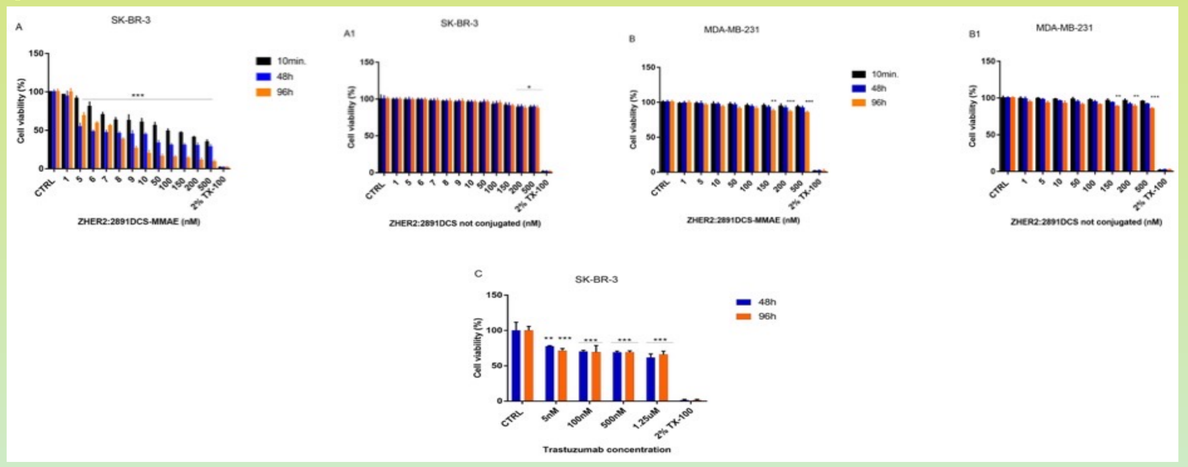

Fig. 2

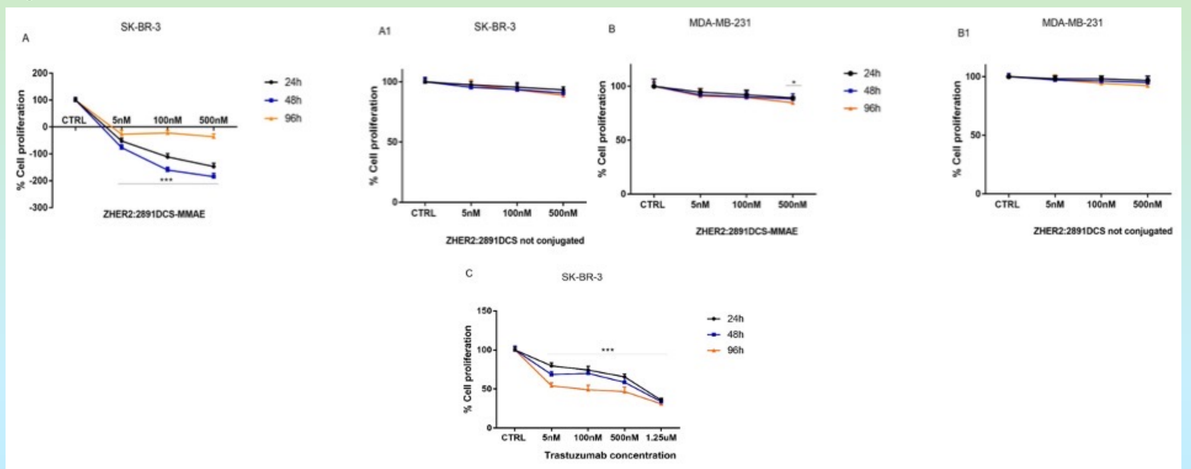

Fig. 3

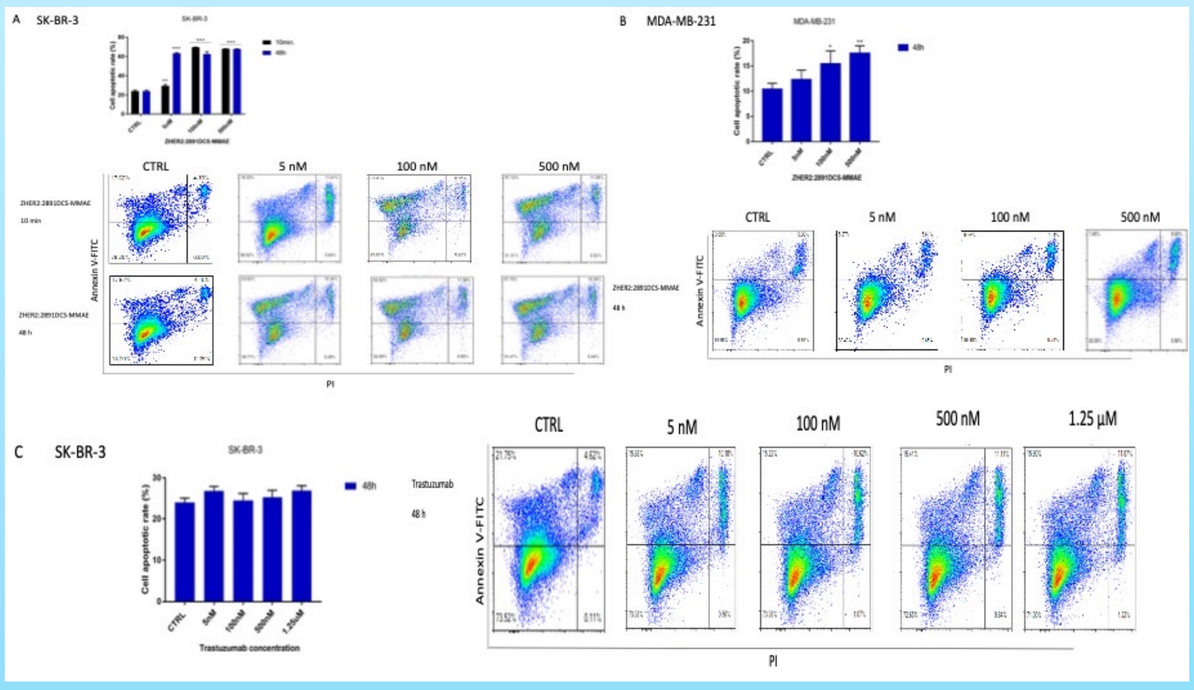

Fig.1 In vitro cell viability assay. SK-BR-3 (panel A, A1, C) and MDA-MB-231 cells (panel B,B1) were treated with increasing concentrations (from 1 to 500 nM) of $Z_{HER2:2891}$DCS-MMAE and not conjugated and with increasing concentrations of trastuzumab (from 5 nM to 1.25 µM). The cytotoxicity was measured using the MTT assay after 10 min followed by drugs removal and an additional 48 h of incubation in medium alone (black bar) or for 48 and 96 hours of continuous exposure to treatments (blue and orange bars, respectively). The p-value was determined by one-way ANOVA test with Dunnett post hoc test and considered significant for $p < 0.05$*, $p < 0.01$**, $p < 0.001$ *** compared with control. Data are presented as mean ± SEM.
Fig.2 Cell proliferation assay. The effect on cell growth of trastuzumab and $Z_{HER2:2891}$DCS-MMAE and not conjugated, was tested in SK-BR-3 (Panel A, A1, C) and MDA-MB-231 cells (Panel B and B1). Cells were treated with the substances for 24, 48, and 96 hours. Cells number was counted with the Coulter Counter. The p-value was determined by one-way ANOVA test with Dunnett post hoc test and considered significant for $p < 0.05$*, $p < 0.001$ *** compared to control. Data are presented as mean ± SEM.
Fig.3 Detection of cell death induced by $Z_{HER2:2891}$DCS-MMAE and trastuzumab treatments. The effect of cellular death induced by $Z_{HER2:2891}$DCS-MMAE and trastuzumab, was tested in SK-BR-3 (Panel A, C) and MDA-MB-231 cells (Panel B) by flow cytometry. The top left quadrant represents the percentage of early apoptotic cells (Annexin V^+/PI^-), the top right quadrant is the percentage of late apoptotic cells (Annexin V^+/PI^+), Whereas the bottom left quadrant represents the percentage of live cells (Annexin V^-/PI^-), and the bottom right quadrant represents the percentage of necrotic cells (Annexin V^+/PI^+). Cells were treated with the substances for 10 min followed by drug removal and an additional 48 h of incubation in medium alone (black bar) or for 48 h of continuous exposure to treatments (blue bar). The p-value was determined by one-way ANOVA test with Dunnett post hoc test and considered significant for $p < 0.05$*, $p < 0.01$**, $p < 0.001$ *** compared to control. Data are presented as mean ± SEM.

References

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