Addressing liver fibrosis by TRAIL targetied to hepatic stellate cells

Arabpour, Mohammad

Chapter 5

Targeting human carcinoma cells by receptor-specific TRAIL and Histone acetyl transferase / Deacetylase inhibitor regimens drives enhanced killing efficiency

Mohammad Arabpour [1], Wim J. Quax [2], Hidde J. Haisma [1]

1. Department of Pharmaceutical Gene Modulation, University of Groningen, Groningen, The Netherlands
2. Department of Pharmaceutical Biology, University of Groningen, Groningen, The Netherlands

Abstract

Background Epigenetic events, including histone acetylation modifications, have been demonstrated to have critical roles in the therapeutic outcome of Tumor Necrosis Factor Related Apoptosis-Inducing Ligand (TRAIL) application in eliminating malignant tumors. Histone acetyl transferase and deacetylase (HDAC and HAT) enzymes are known to play the central role in the process of histone acetylation modification. In the present study, we investigated the potential application of a combining novel HDAC or HAT inhibitors with TRAIL variants in killing human carcinoma cells.

Methods A panel of different carcinoma cell lines (colon, lung and hepatocellular carcinoma) was treated with different TRAIL variants in presence of different HDAC or HAT inhibitors to evaluate its effect on the viability of these cell lines.

Result *In vitro* treatment of human carcinoma cells with different receptor specific and wt TRAIL variants induced a reduction in viability with DR4 specific TRAIL being the most efficient. However, a significant impairment in cell viability was observed during administration of TRAIL in presence of HDAC inhibitor SAHA or HAT inhibitor C646.

Conclusion Here, we present evidence for the successful application of the receptor specific TRAIL variants in the elimination of human carcinoma cells via simultaneous inhibition HAT or HDAC enzymes. The combined HAT or HDAC inhibitor and TRAIL variant regimen may thus represent a new therapeutic compound against different human carcinoma.

Introduction

Since TRAIL protein has been introduced to the field of tumor therapy it has raised many hopes for specific elimination of tumor cells. The application of TRAIL to induce apoptosis in tumors is mainly grounded on up-regulation of TRAIL receptors in tumor cells versus normal cells. TRAIL has two dedicated receptors (DR5 and DR4) that signal apoptosis via binding to TRAIL agonists. However, up-regulation of decoy receptors (DCR1 and DCR2) in tumor cells could lead to abortive signaling and impaired apoptosis. This later is considered the primary source of TRAIL resistance and tumor evasion. To address this problem receptor specific agonist TRAIL has already been introduced both in the form of type specific mutant TRAIL agonists or monoclonal antibody against specific TRAIL receptors DR4 and DR5 [1][2][3]. Dynamic of wild type (wt) occurring TRAIL and mutant TRAIL interaction with their receptors have already been well characterized[4]. Receptor specific agonists could reduce the decoy receptor-mediated antagonism, hence a cumulative effect by using receptor specific TRAIL is lowering required administrated dose with possibly fewer side effects that attached to TRAIL adverse effect [4][5]. Epigenetic alterations could also lead to defective apoptotic signaling and develop TRAIL resistance in tumor cells. Histone acetylation is the result of the balance between the activity of histone deacetylases (HDAC) and histone acetyl transferases (HAT). Extent of histone acetylation determines the extent of chromatin relaxation hence plays a major role in the regulation of gene expression. Deregulation of HAT or HDAC in tumor cells is associated with the failure to undergo apoptosis in cancer cells. Through chromatin condensation HDAC could

repress the expression of tumor suppressor and pro-apoptotic genes and confer resistance to apoptosis [6][7][8] , whereas HATs affect the chromatin remodeling and can promote expression of cancer related proto-oncogenes in cancer[9][10][11]. Extensive studies have demonstrated synergistic effects of HDAC inhibitors and TRAIL on apoptosis [6][7][8]. Unlike HDAC inhibitors, the interactive role of HAT inhibitors and TRAIL on apoptosis have been less characterized. In this study we investigate the potential application of TRAIL variants in combination with HDAC and HAT inhibitors to eliminate cancer cells.

Methods and materials

Cells and cultures

SW948 [SW-948] (ATCC CCL-237) Dukes' type C, grade III, colorectal adenocarcinoma, was cultured in Leibovitz's L-15 Medium (Sigma-Aldrich) supplemented with 10% Fetal calf serum. NCI-H460 [H460] (ATCC HTB-177) Human lung carcinoma cell line cultured in RPMI-1640 (Sigma-Aldrich) Medium supplemented with 10 % fetal calf serum. HepG2 [HEPG2] (ATCC HB-8065) hepatocellular carcinoma cell line cultured in DMEM high glutamine (Glutamax) (Sigma-Aldrich) supplemented with 10 % fetal calf serum. Huh-7 cell line cultured in DMEM high glutamine (Glutamax) (Sigma-Aldrich) supplemented with 10 % fetal calf serum. All the cells were grown in 37°C in presence of 5 % Co2.

Chemicals

Receptor specific or wild type TRAIL were produced and purified from prokaryotic expression as previously described [1][2][3]. Crystal violet solution (Sigma-

Aldrich), HAT inhibitors C646 Ca t# S7152 and MG149 cat # S7476 (Selleckchem). HDAC inhibitors; SAHA **N**-hydroxy-N′-phenyl-octanediamide, Suberoylanilide hydroxamic acid, Vorinostat, cat #SML0061 and MS-275 A HDAC1 and HDAC3 inhibitor Synonym: **3-**pyridinylmethyl[[4-[[(2-aminophenyl) amino] carbonyl] phenyl] methyl] carbamate, MS-275 (Entinostat, SNDX-275), N-(2-Aminophenyl) -4- [N -(pyridine-3 etylmethoxycarbonyl) naminomethyl] benzamide cat # EPS002 (Sigma-Aldrich).

Crystal violet viability assay

Then, 100 µl of a cell suspension 10 4 cells/ml for mentioned cells were gently introduced into each well from 96 well plates. After being kept undisturbed at room temperature for 20 min to allow the cells to sediment, the plates were transferred to a CO2 incubator and cultured for 2 days. The cells then were treated with 100 µl of medium containing different combination of wt and receptor specific TRAIL in presence or absence of HAT or HDAC inhibitors for hours. For cell fixation each well received 50 ul glutaraldehyde (25 %) and was left for at least 20 min. After being washed with water, the plates were stained with 0.4 % crystal violet solution in methanol for 30 min. Absorbance at 590 nm was measured by an automatic microtiter plate reader. Average absorbance of the control wells, which received no chemical, was regarded as 100 %, and the percentage of cell growth in each well was calculated.

Results

HAT and HAC inhibitors markedly potentiate TRAIL lethality in colon carcinoma cell line

To evaluate the effect of receptor specific TRAIL, different amounts from receptor specific or wt TRAILs were added to SW948 cells and the viability of cells was determined 24 hours later by the CVS assay. SW948 cells showed slight decrease in viability occurred in the presence of different TRAIL variants at sub-lethal concentration. Also comparison between different concentrations of SAHA and Entinostat showed SAHA displays a greater potential in reducing viability of SW948 cells (Fig 1). Co-treatment of SW948 cells with different type of TRAIL and HADAC inhibitors significantly increased SW948 sensitivity to apoptosis (70 % and 90 % in order for Entinostat and HDAC) at the sub-lethal concentration (10ng/ml) of TRAIL variants (Fig. 1). We next evaluated the contribution of HAT inhibitors with respect to their effect on increasing SW948 cells sensitivity towards TRAIL variants. Measurement of viability in SW948 cells treated with TRAIL variants (10ng/ml) in presence of MIG 149 and C646 showed a decrease of 40 % and 80 % respectively in compare with cells treated only with TRAIL variants (Fig. 2).

Potentiation of apoptosis in H460 lung carcinoma exposed to TRAIL and HAT/HDAC inhibitors

To further investigate the apoptosis-inducing activities of different TRAIL variants, H460 lung carcinoma cells were incubated with PBS or the aforementioned TRAIL variants proteins. Consistent with cell viability results, only DR4 specific TRAIL induced robust apoptosis of H460 cells (90 %), whereas wt and DR5 specific TRAIL

were less effective in reducing viability at lower concentration of 10 ng/µl (Fig. 3). In marked contrast to results obtained following exclusive treatment of cells with HDAC inhibitor drugs, simultaneous exposure of cells to a sub-toxic concentration of TRAIL (10 ng/ml) in conjunction with 1uM Entinostat or SAHA resulted in a very dramatic decrease in cell survival (Fig. 3). To determine whether activators of the extrinsic pathway could similarly enhance HAT inhibitor-associated lethality, H460 cells were simultaneously exposed to either C646 or MG149 in combination with different TRAIL variants. As shown in Fig. 4, while DR5 receptor specific TRAIL alone was minimally toxic, co-administration of HAT inhibitors and in particular C646 resulted in marked increase in the extent of cell survival loss. The maximum cytotoxic effect was achieved by combining 10 uM C646 and TRAIL (10 ng/ml) (Fig .4).

Co-administration of TRAIL with HDAC inhibitors potently induces apoptosis in human hepatocellular carcinoma cell lines

To determine whether the previous findings were restricted to colon or lung carcinoma cells, parallel studies were performed using human hepatocellular carcinoma cell line; Huh-7. Cells were exposed for 24 h to 1 or 5 -mM Entinostat or SAHA in presence of 10ng or 100-ng/ml TRAIL variants after which cell death was assayed. Responses to HDAC inhibitors given individually varied between the administered drugs with Huh-7 cells showed greater sensitivity to SAHA in compare to Entinostat. Simultaneous exposure of cells to TRAIL in conjunction with HDAC inhibitors resulted in a clear increase in apoptosis, comparable with results obtained in cells only treated with SAHA. This effect however was most pronounced for DR4

TRAIL ligand treated cells (Fig. 5). In the same way administration of HAT inhibitors showed that Huh-7 cells showed greater sensitivity to C646 in compare with MG149. Co-administration of C646 and DR4 TRAIL promises to be the most effective combination in inducing apoptosis in Huh-7 cells (Fig. 6).

Conclusion

TRAIL agonists are vastly used to kill malignant cells that express TRAIL receptors. The extrinsic pathway of apoptosis is initiated by the binding of death receptors, including Fas (CD59), TNF receptor-1 (TNFR-1) and TRAIL receptors (DR-4 and -5) to their correspondent ligands and the subsequent activation of caspase-8 and caspase-10[12]. However, usefulness of TRAIL in inducing apoptosis could be hampered by the resistance of some cancer cells to TRAIL. Of all known mechanism of TRAIL resistance epigenetic events, including histone acetylation modifications, have been demonstrated to have critical roles in developing resistance toward TRAIL through different mechanisms.

The present findings indicate that simultaneous administration of TRAIL with HDAC inhibitors induces apoptosis through sensitization of malignant cells to TRAIL effect. Treatment a panel of carcinoma cell lines (Lung, colon and hepatocellular) with TRAIL variants in presence of SAHA drastically enhance cells sensitivity to apoptosis. In contrast, application of Entinostat did not significantly increase TRAIL sensitivity. Importantly the results emphasize SAHA sensitization of TRAIL-resistant cells achieved with the lowest concentration of SAHA and

TRAIL that otherwise were not effective alone. However, the main question remains as to how combined treatment with SAHA and TRAIL resulted in such a significant impairment in cell survival. In fact the cooperative effects of SAHA and TRAIL could be interpreted in several levels; HDAC inhibitors can upregulate the expression of death receptors and their ligands in transformed cells, yet not in normal cells [13][14]. TRAIL receptors DR-4 and DR-5 were induced both *in vivo* [14][15] and *in vitro* [16] by different types of HDAC inhibitors[17][18][19][20][21]. On the basis of our results, receptor specificity of TRAIL variants did not significantly contribute to their killing effect in presence of SAHA. This later could indicate HDAC inhibitors mechanism of action in sensitizing cells is independent of a specific TRAIL receptor up-regulation. HDAC inhibitors could also amplify TRAIL killing effect in cancer cells through other mechanisms including; up-regulation of caspase-8, down-regulation of anti-apoptotic molecules like cFLIP and Bcl-2 proteins (e.g., Bcl-2, Bcl-XL, and Mcl-1), increase in pro-apoptotic Bcl-2 proteins (e.g., Bid, Bim, and Bax), and re-distribution of TRAIL receptors in lipid rafts on the surface of targeted cells [22][23][24] . However, the exact mechanism of this phenomenon needs to be further investigated. Unlike HDAC inhibitors, less extensive studies have been carried out to unravel the role of HAT inhibitors in TRAIL-induced apoptosis. Selective inhibition of HAT inhibits the DNA damage response in malignant cell lines [11]. Here we evaluate cytotoxicity induced by pretreatment of multiple myeloma cells with C646 or MG149 followed by TRAIL using CVS cell proliferation assay. Whereas MG149, low concentration of C646 (HAT inhibitor)

augments TRAIL-induced Cytotoxicity in multiple carcinoma cell lines. Indirect evidence suggests some level of regulation on TRAIL receptor expression due to effect of HAT inhibitors. For instance P53 a key factor that increases as a result of inhibiting P300/BCP (histone acetyltransferase) could upregulate DR5 receptor in conjunction with NF-κB[15][25][26][27]. Selective inhibition of P300 HAT via C646 HAT inhibitor could also decrease expression of a series of genes including Ubiquitin-like with PHD and ring finger domains (UHRF1) (responsible for G1/S transition and p53-dependent DNA damage checkpoint), DEP domain containing (DEPDC1) (responsible for inhibition of apoptosis) up to 14 times; while increasing the expression of P53 gene that is responsible for response to diverse cellular stresses and apoptosis senescence 1.2 times [28][29][30]. In conclusion, present evidence indicates that TRAIL in conjunction with HDAC or HAT inhibitors constitutes a potent apoptotic stimulus in human carcinoma cells. Previous studies have demonstrated that co-administration of HDAC inhibitor with TRAIL enhance the antitumor activity of TRAIL [6][7][8]. The present results suggest that similar interactions occur in human carcinoma cells exposed to TRAIL in combination with HAT inhibitors. Given that there is a growing interest in combined regimen for more efficient application of TRAIL in anti-tumor therapy, the current finding is the first to show a significant anti-tumor concept of combining TRAIL variants with HAT inhibitors, which warrants further detailed studies *in vivo*.

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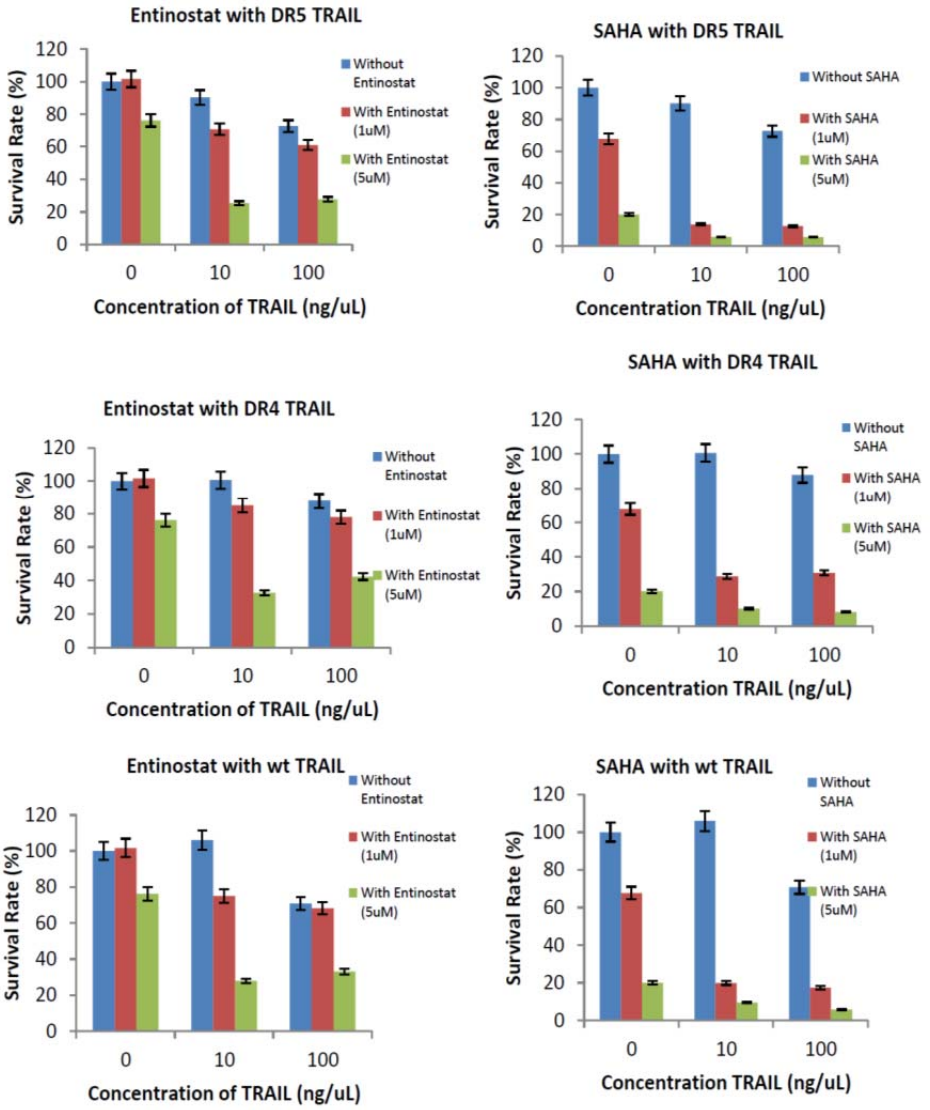

Figure 1: Simultaneous application of histone deacetylase inhibitor and TRAIL enhances the efficiency of TRAIL variants in SW948 colon carcinoma cell line.

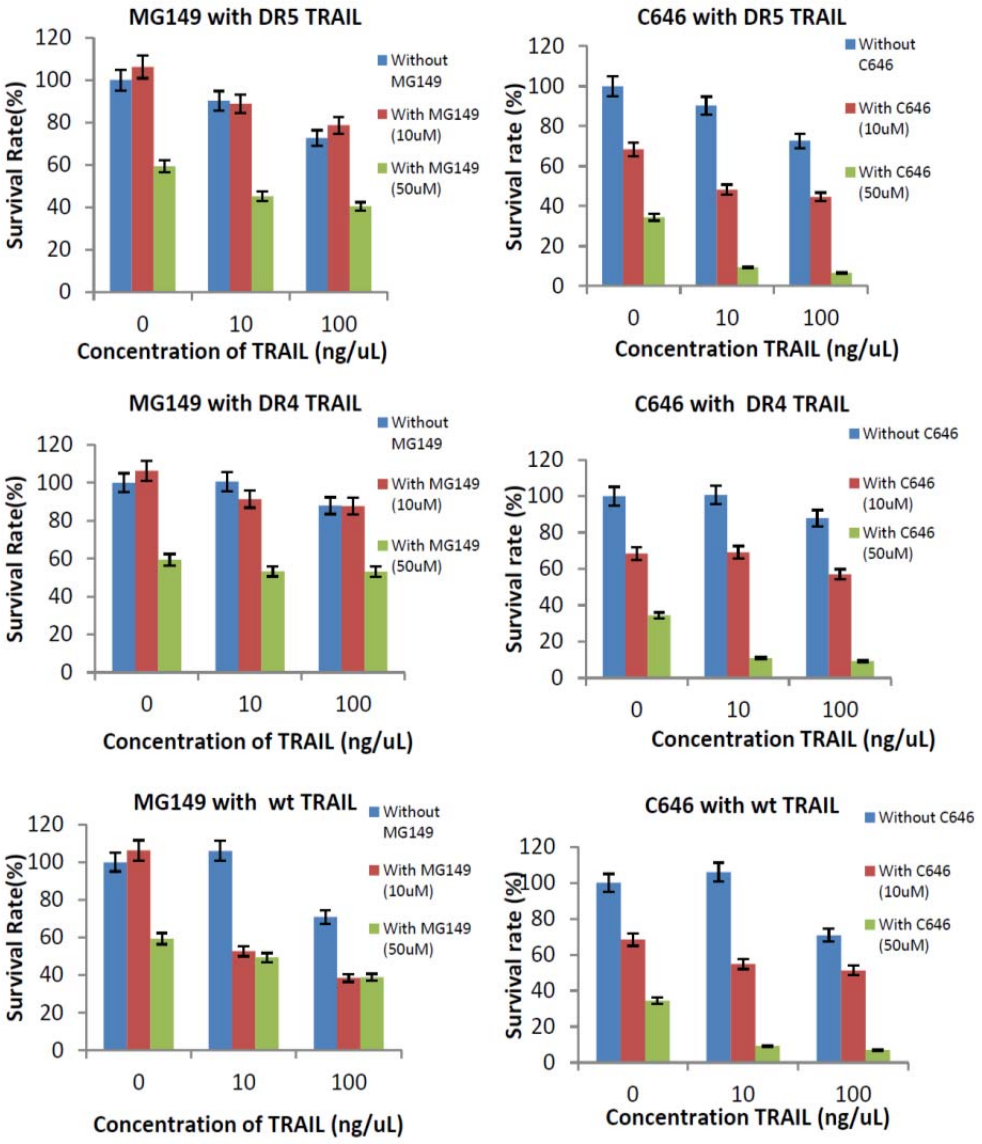

Figure 2: Simultaneous application of HAT inhibitor and TRAIL enhance the efficiency of TRAIL variants in SW948 colon carcinoma cell line.

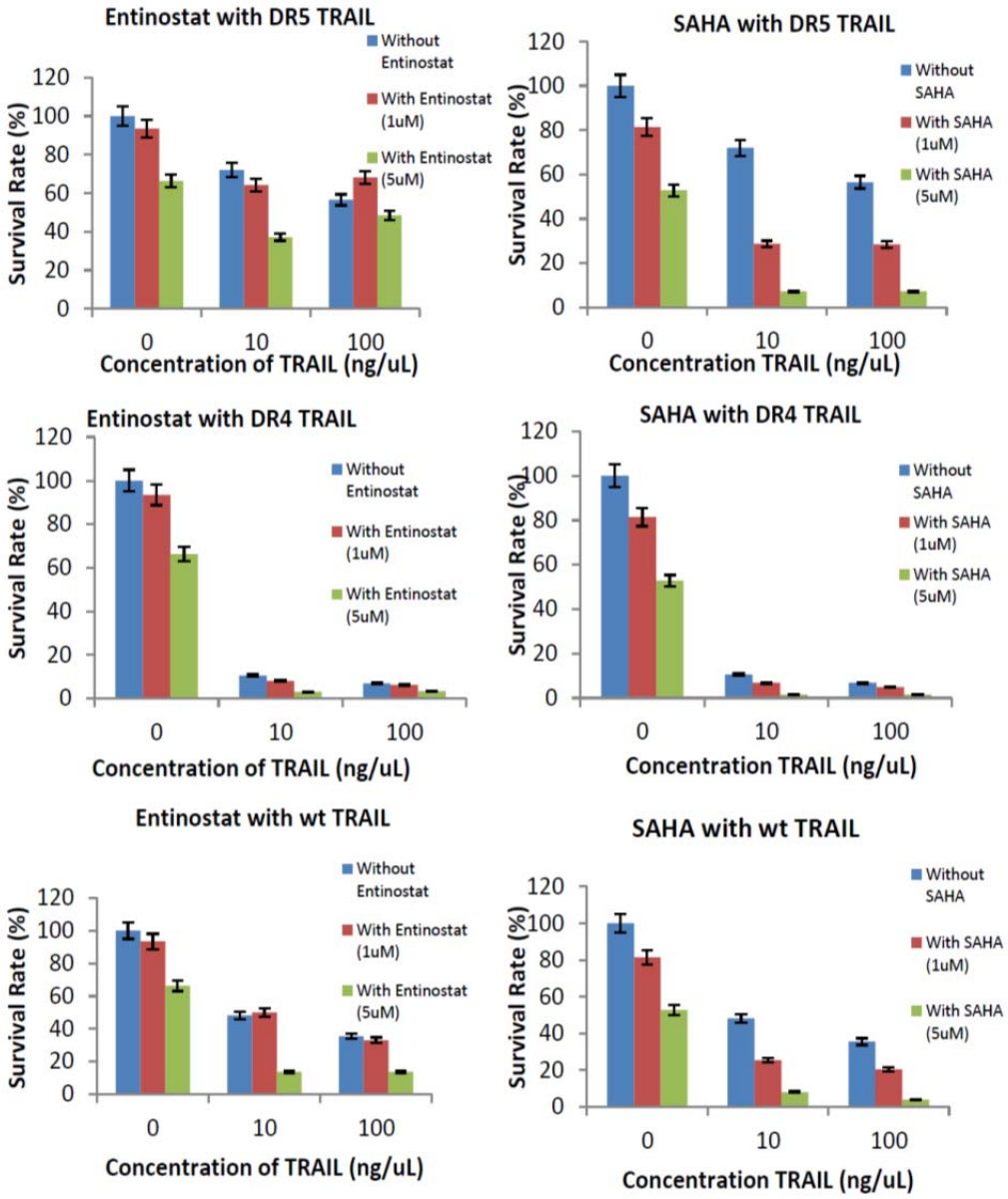

Figure 3: Simultaneous application of histone deacetylase inhibitor and TRAIL enhance the efficiency of TRAIL variants in H460 (lung carcinoma) cell line.

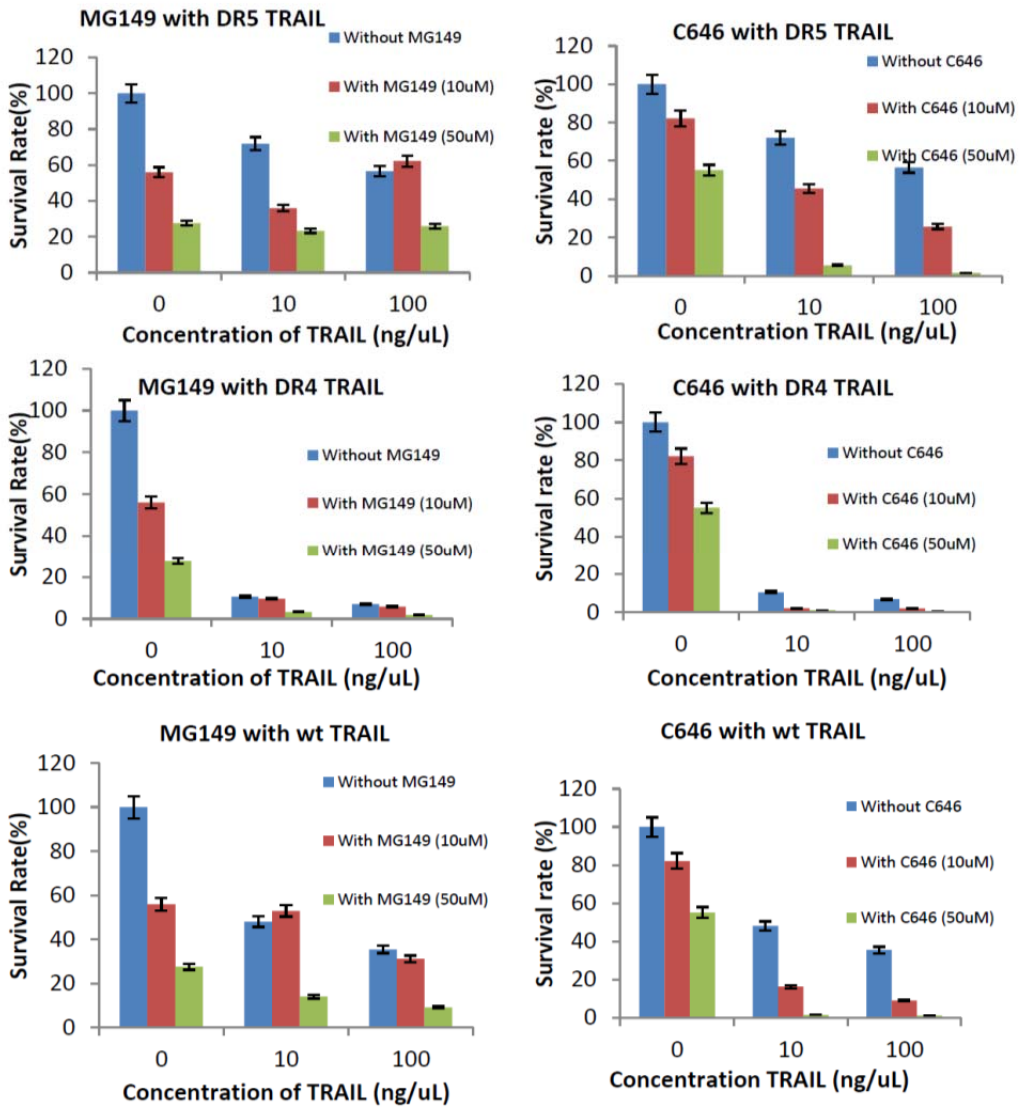

Figure 4: Simultaneous application of histone acetylase inhibitor and TRAIL enhance the efficiency of TRAIL variants in H460 cell line.

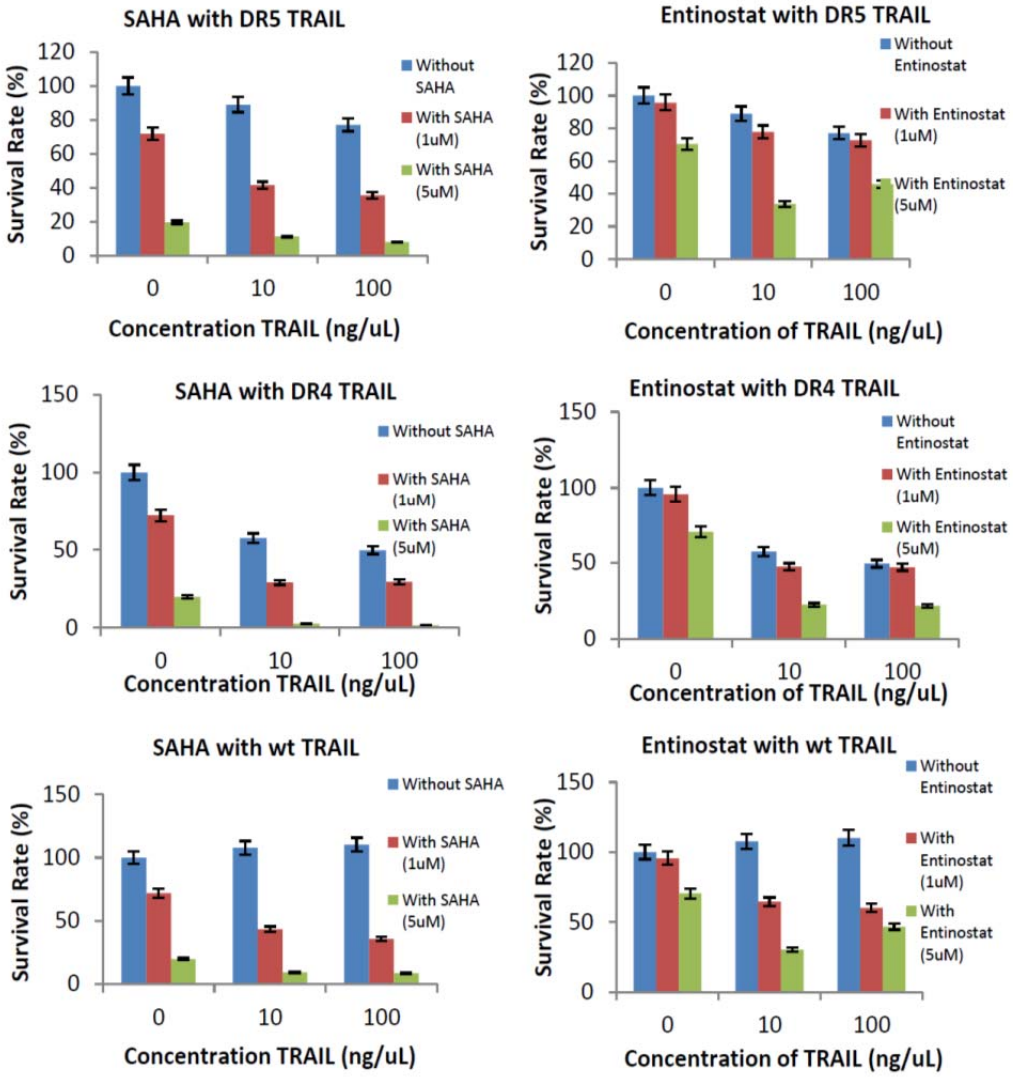

Figure 5: Simultaneous application of histone deacetylase inhibitor and TRAIL enhance the efficiency of TRAIL variants in Huh-7 (hepatocellular carcinoma) cell line.

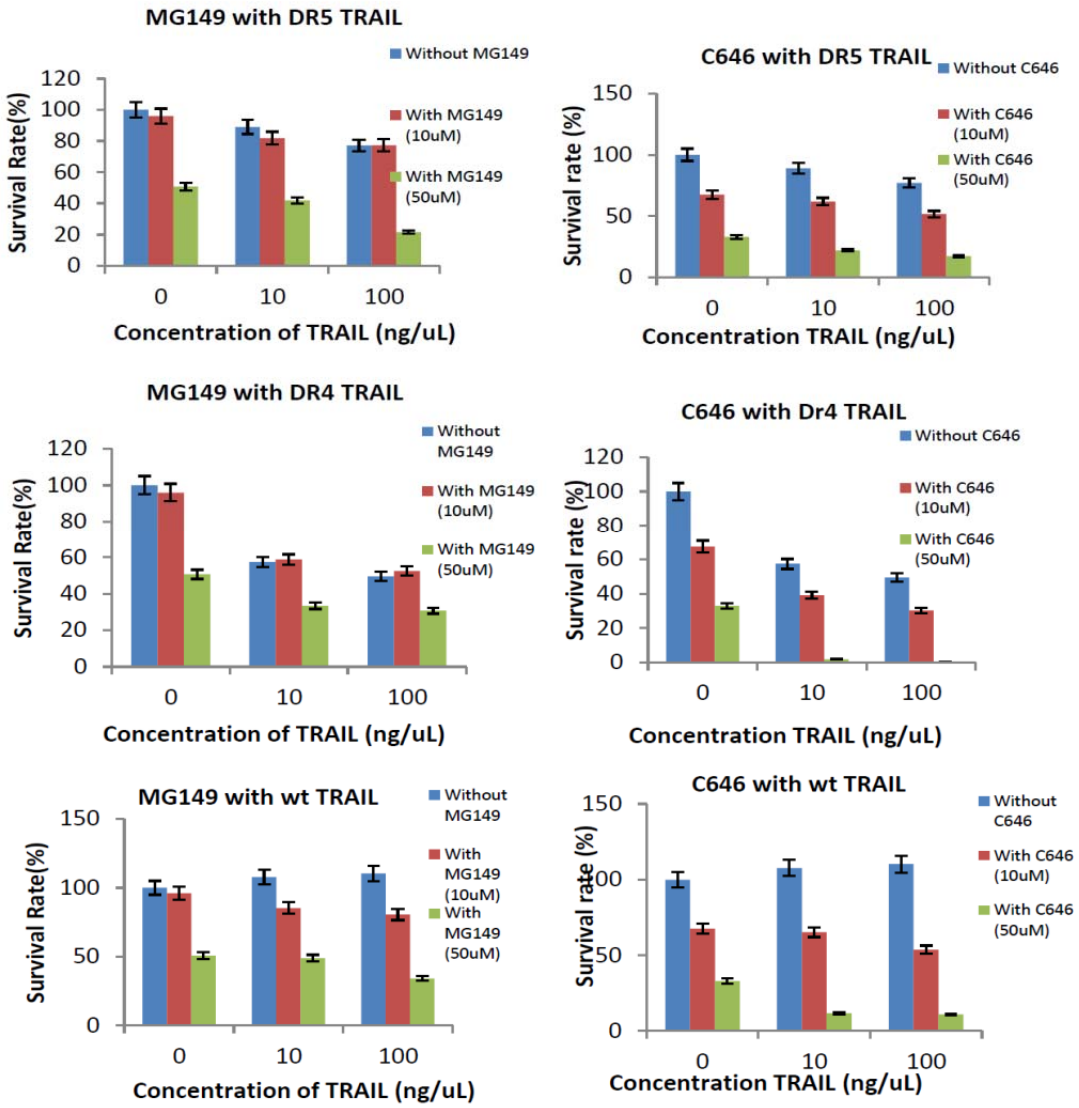

Figure 6: Simultaneous application of histone acetylase inhibitor and TRAIL enhance the efficiency of TRAIL variants in Huh-7 cell line.