Effect on tumor cells of selective inhibitor of ER stress response

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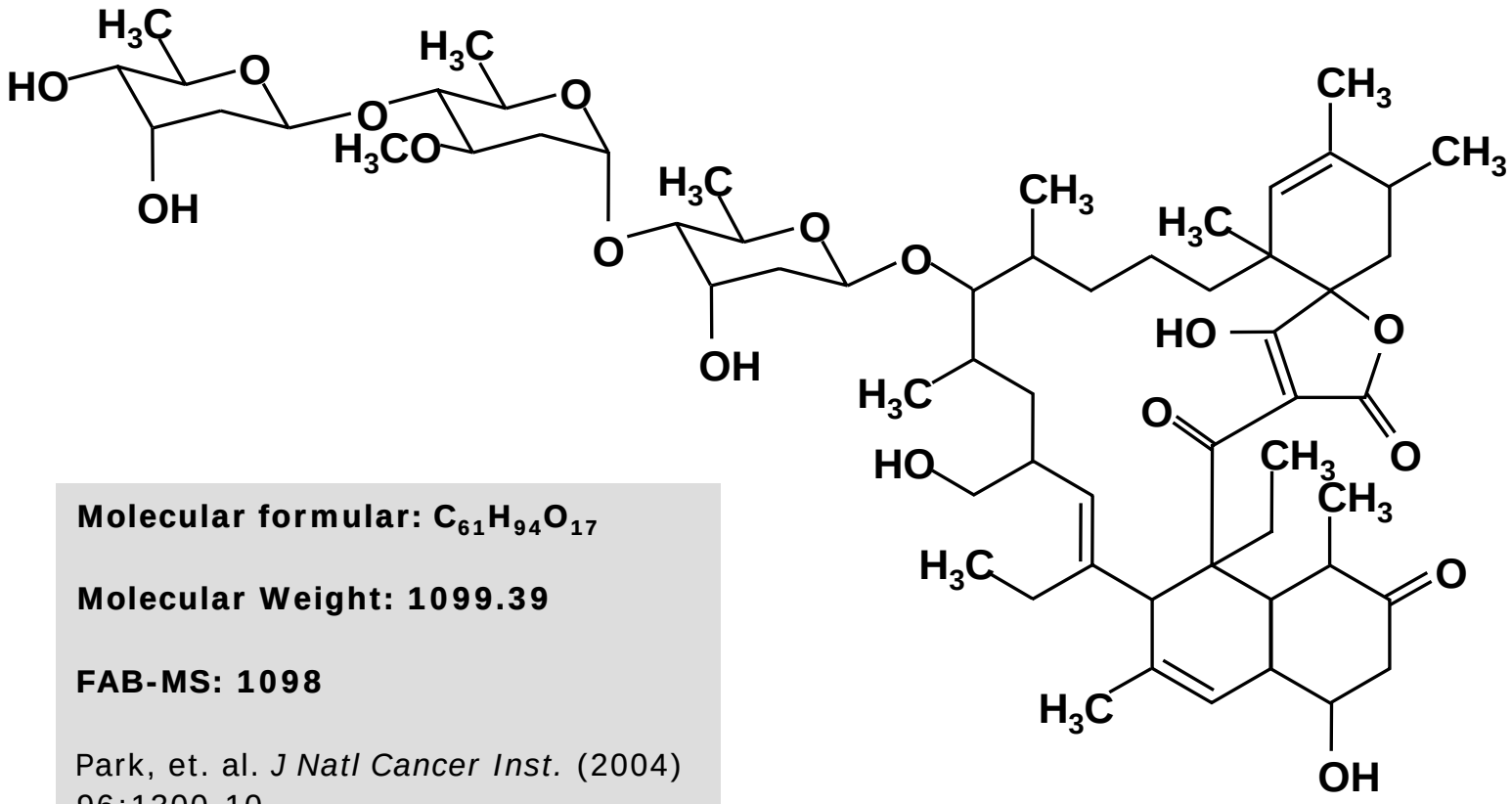
Introduction

In poorly vascularized solid tumors, cancer cells are exposed to micro-environmental stresses, such as glucose deprivation. Glucose deprivation disrupt protein folding in the endoplasmic reticulum (ER), and activate the **unfolded protein response (UPR)** which influences cell survival during microenvironmental stress. Therefore, the UPR to the unique micro-environmental conditions would be exploited for the selective killing of solid tumor cells.

Here, we demonstrate that VST, a novel compound, disrupts the network of UPR transcriptional activation during glucose deprivation. Consequently, VST exhibits a striking selective cytotoxicity towards glucose-starved tumor cells. We also show the *in vivo* potential of the antitumor activity of VST.

Chemical: Versipelostatin (VST)



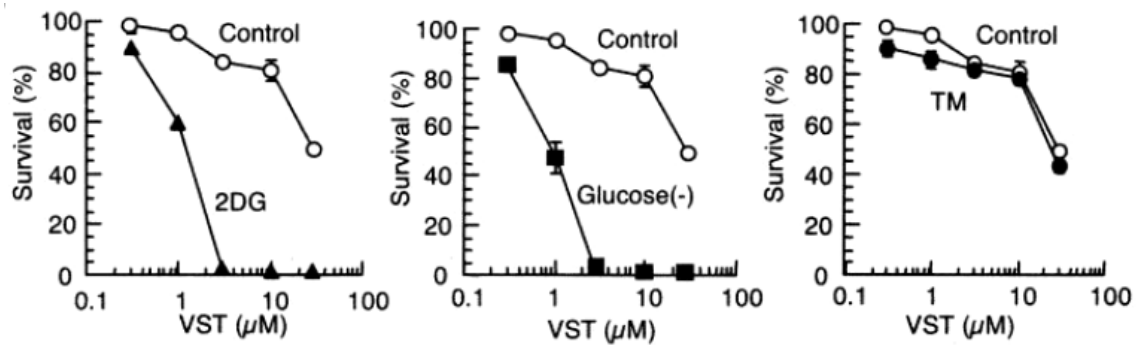
Molecular formular: $C_{61}H_{94}O_{17}$

Molecular Weight: 1099.39

FAB-MS: 1098

Park, et. al. *J Natl Cancer Inst.* (2004) 96:1300-10.

Result 1.
Glucose-dependent effect of VST on cell viability.

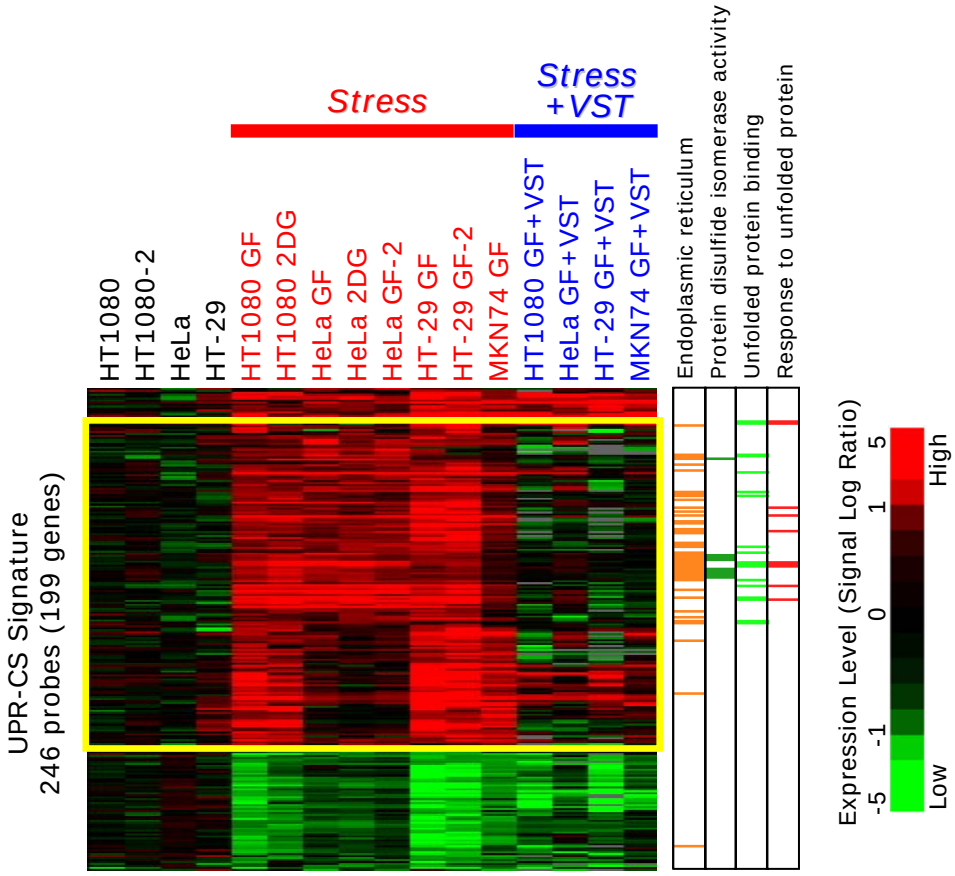


Colony formation analysis of HT-29 cells after a 24-hour VST treatment under normal growth (control) or stress conditions, as indicated. 2DG = 2-deoxyglucose (20 mM); TM = tunicamycin (5 μg/ml). Data (mean values with 95% confidence intervals in triplicate determinations) are representative of at least two independent experiments.

Under normal growth conditions, 24 hours of VST treatment of HT-29 cells had only a weak effect on cell viability, with approximately 30 *M* VST required to inhibit colony formation by 50% (IC50). By contrast, VST was highly toxic in cells exposed to glucose free or 2DG-containing medium, resulting in approximately 30-fold lower IC50 (1 *M*). Under hypoglycemic conditions, the cytotoxic activity of VST was associated with the inhibition of GRP expression (*Result 1, 2*). By contrast, there was no consistent combined effect of VST with the chemical stressor TM.

Result 2.

Stress-induced expression was widely inhibited by VST.

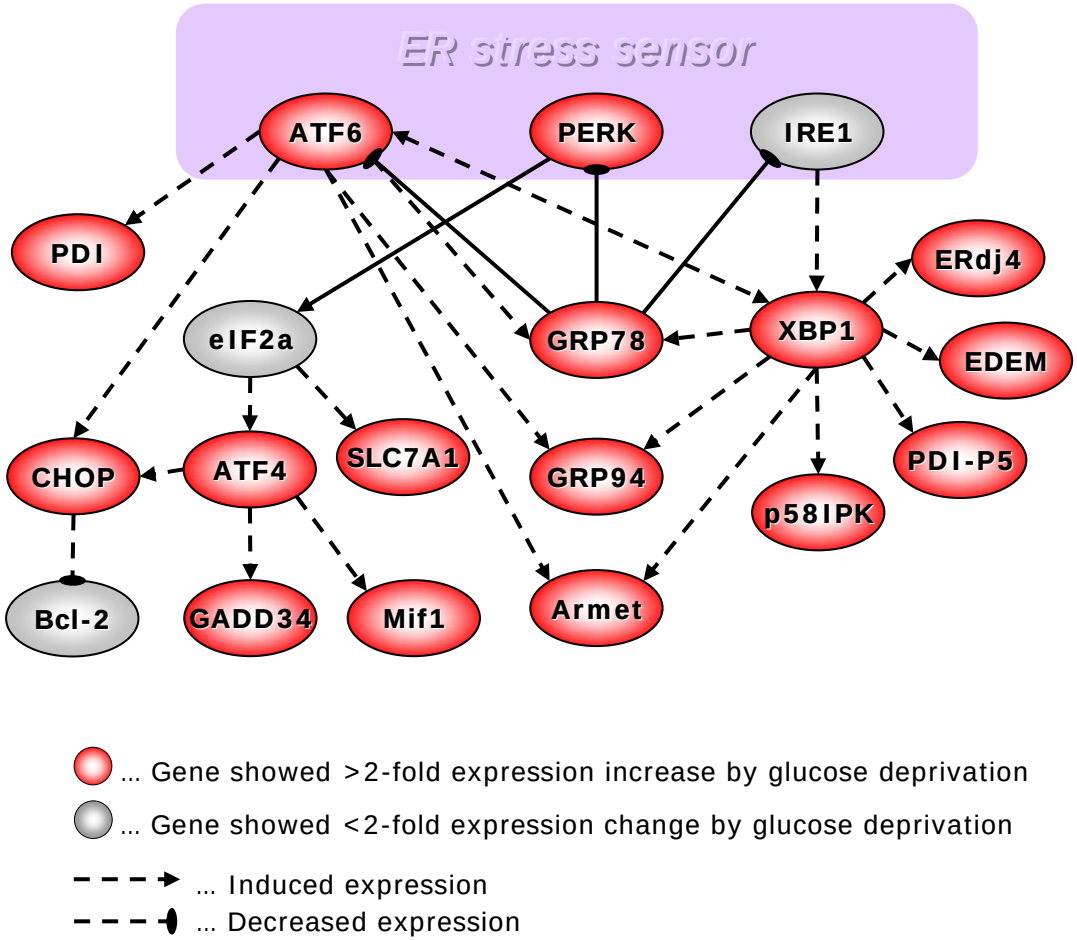


Using microarray analysis, we selected 199 genes, named "UPR-CS (Cell Survival) signature", which remarkably changed their expression by glucose deprivation. Moreover, it is showed that about 90% of glucose deprivation-inducible gene expression (*enclosed by yellow line*) was inhibited by VST.

Gene Ontology showed that VST-sensitive genes have ER stress-related GO term, such as "ER", "protein disulfide isomerase activity", "unfolded protein binding", or "response to unfolded protein".

Microarray analysis of 4 cell lines cultured for 18 hours under normal growth (*black*) or stress condition (*red*) as indicated. 2DG = 2-deoxyglucose (HT1080, 10 mM; HeLa, 5 mM); GF = glucose free. VST (HT-29, 10 μM; HT1080, 3 μM; HeLa and MKN74, 1 μM) was treated for 18 hours under glucose free (*blue*). cRNA targets were prepared from 5 μg of total RNA, and hybridized to GeneChip Human Genome U133 Plus 2.0 arrays (Affymetrix). The 246 probes (vertical axis) sorted by cluster analysis displayed with 16 samples (horizontal axis).

Result 3.
ER stress signaling was repressed by VST.



Pathway analysis using KeyMolNet software (IMMD Inc.) shows that the UPR-CS signature included genes which acted in ATF4/ATF6/ IRE1 signaling pathways.

Microarray analysis showed that, in ATF4/ATF6/IRE1 signaling pathways, the induction of expression by glucose deprivation (red circle) was extensively repressed by VST.

Conclusion

■ VST shows highly selective cytotoxicity to glucose-deprived tumor cells that is associated with inhibition of the UPR.

■ VST inhibited expression of the UPR target genes GRP78 and GRP94, repressed the production of the UPR transcriptional activators XBP1 and ATF4 during glucose deprivation, and suppressed the activation of ATF4/ATF6/ IRE1 signaling pathway stimulated by glucose deprivation.

■ VST showed in vivo antitumor activity at well-tolerated doses.