Introduction

Cervical cancer, the sixth most deadly cancer in the world, is primarily caused by HPV infections. E6 and E7 (HPV oncoproteins) transform normal cells by inducing the degradation of p53 and retinoblastoma protein, respectively, making them highly resistant to radiation and chemotherapy.

Induction of apoptosis in transformed cells is a key strategy in successfully treating HPV-induced cervical cancer.

TRAIL has been shown to selectively induce apoptosis in cancer cells by binding DR4/DR5 and activating extrinsic pathways of apoptosis (Figure 2).

Certain cervical cancers, such as the cultured cell line SiHa, are remarkably resistant to TRAIL.

Sanguinarine, a molecule from the plant Sanguinaria canadensis, has been shown to selectively induce apoptosis in cancer cells via multiple cell death pathways (Figures 2 & 3).

Hypothosis: In primary effusion lymphoma cells, sanguinarine has been shown to upregulate DR5 expression and insertion on the cell membrane via ROS (3). In breast cancer cells, it exhibits synergistic activity with TRAIL. We hypothesize that sanguinarine can induce upregulation of DR4/5 and sensitize cervical cancer cells to TRAIL, resulting in cell death by apoptosis (Figure 4).

Materials and Methods

Methods: Cultured SiHa cells were exposed to sub-lethal doses of sanguinarine in combination with TRAIL. Cell viability changes were assessed, and induction of apoptosis was further investigated by assays for caspase activation and the production of reactive oxygen species. Flow cytometry was performed to measure upregulation of death receptors 4/5.

Results: Sanguinarine treatment led to a significant increase in oxidative stress and the upregulation of death receptors in SiHa cells. When combined with TRAIL, sanguinarine led to the induction of apoptosis via activation of the caspase cascade and resulted in a significant reduction in cell viability.

Discussion

- Sanguinarine induces cell death (IC50 of 1.75µM) while TRAIL fails to induce cell death, even at high concentrations (Fig. 6 & 7).
- Preliminary FACS analysis indicates upregulation of death receptor 4 and 5 after treatment with sanguinarine (Fig. 8 & 9).
- At sublethal concentrations, sanguinarine sensitizes SiHa cells to TRAIL, resulting in cell death (Fig. 10).
- Caspase assays and morphological observation, confirm that cell death is, in part, due to apoptosis (Fig. 13, Fig. 15 & 16).
- ROS analysis suggests sanguinarine upregulates ROS production in SiHa (Fig. 14).
- The interaction between sanguinarine and TRAIL on SiHa cells is promising for the treatment of cervical, and possibly other, HPV-induced cancers.

Further analyze specific molecular components of the pathway that leads to apoptosis and use mouse models to test the synthetic lethal interaction between sanguinarine and TRAIL in vivo.

References


3. Cervical cancer (250ng/mL) has been shown to selectively induce apoptosis in cancer cells by binding to death receptors and activating extrinsic pathways for apoptosis. However, certain cervical cancers, such as the cultured cell line SiHa, are remarkably resistant to TRAIL. In this study, we have explored the use of sanguinarine, an extract from the plant Sanguinaria canadensis, to sensitize SiHa cells to TRAIL. Sanguinarine has been shown to induce apoptosis in cancer cells by activating multiple cell death pathways, including the upregulation of death receptors via reactive oxygen species.

Hypothosis: Since sanguinarine may lead to oxidative stress and upregulation of death receptors, we hypothesize that it can potentially sensitize SiHa cells to TRAIL and lead to apoptosis.

Methods: Cultured SiHa cells were exposed to sub-lethal doses of sanguinarine in combination with TRAIL. Cell viability changes were assessed, and induction of apoptosis was further investigated by assays for caspase activation and the production of reactive oxygen species. Flow cytometry was performed to measure upregulation of death receptors 4/5.

Results: Sanguinarine treatment led to a significant increase in oxidative stress and the upregulation of death receptors in SiHa cells. When combined with TRAIL, sanguinarine led to the induction of apoptosis via activation of the caspase cascade and resulted in a significant reduction in cell viability.

Conclusion: The observed synergistic effect of sanguinarine and TRAIL on SiHa cells is promising for the treatment of cervical, and possibly other, HPV-induced cancers. Oxidative stress caused by sanguinarine seems to play a central role in this synergy. The molecular pathways that link reactive oxygen species and the possible upregulation of death receptors needs further investigation. This knowledge will enable us to devise more effective treatments for those who suffer with this devastating disease.

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