The radiosensitizing effect of Chinese herbal medicines in cultured human breast cancer cells

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The incidence and mortality of breast cancer in Taiwan are increasing year by year. Breast conserving therapy including conserved mastectomy combined with radiation therapy is the contemporary choice of treatment. Radiosensitizers are often used in radiation therapy program to augment the anti-tumor effect. However, the clinical application of traditional radiosensitizers is limited due to their serious side effects. In this thesis, the radiosensitizing effect of Chinese herbal medicines in cultured breast cancer cells was examined to search for new radiosensitizers with high therapeutic effects but low side effects.

First, the effects of 20 Chinese herbal medicines within the categories of clearing away heat and detoxication as well as promoting circulation and dispelling stagnant blood (such as Artemisia apiacea, Taraxacum mangolicum, Patrinia scabiosaefolia, Viola yedoensis, Hedyotis diffusae, Lonicera japonica, Paeonia suffruticosa, Polygonum cuspidatum, Isatis indigotica, Scutellaria baicalensis, Phellodendron amurense, Savia tatarica,
Carthamus tinctorius, Sparganium stoloniferum, Curcuma zedoaria, Panax pseudo-ginseng var.notoginseng, Curcuma longa, Vaccaria pyramidata, Glycyrrhiza uralensis, and Prunella vulgaris) on cell growth of MDA-MB-231 (p53⁻, Rb⁻, ER⁻) human breast cancer cells were examined. The components of some herbal medicines, such as Epigallocatechin gallate, baicalin, and baicalein, were also examined. Roscovitine, a known radiosensitizer in MDA-MB-231 cells, was used as a positive control. The growth inhibition induced by herbal medicines was measured by MTS assay. Some herbal medicines, such as Curcuma longa, Sparganium stoloniferum, Taraxacum mangolicum, and Isatis indigotica, showed no significant growth inhibition in MDA-MB-231 cells (P>0.05). Some herbal medicine and their components, such as Scutellaria baicalensis, Phellodendron amurense, Vaccaria pyramidata, baicalin, and baicalein, induced significant growth inhibition in a dose-dependent manner in MDA-MB-231 cells (P<0.05). The 50% inhibitory concentrations (IC50s) of roscovitine, baicalin, baicalein, Phellodendron amurense, Vaccaria pyramidata, and Scutellaria baicalensis were 10.3, 13.8, 26.0, 32.3, 36.9 and 207.7 µg/ml, respectively. On the contrary, most of the herbal medicines demonstrated no significant growth inhibition in MCF-7 (p53⁺, Rb⁺, ER⁺) human breast cancer cells by MTS assay, except Phellodendron amurense.

Using MTS assay, these is no significant radiation–induced growth
inhibition observed in both MDA-MB-231 and MCF-7 cells 24, 48, 72, 96 hours after 3 ~ 7 Gy irradiation. In contrast, radiation (2, 3, 4, 5 Gy) exhibited significant dose-related growth inhibition in MDA-MB-231 cells by clonogenic survival assay. The growth inhibition rates of 2, 3, 4, and 5 Gy were 26%, 39%, 54%, and 65%, respectively, as compared with negative control.

The radiosensitizing effects of herbal medicines in MDA-MB-231 cells were examined by clonogenic survival assay. Some herbal medicines, such as *Scutellaria baicalensis*, *Curcuma zedoaria*, *Hedyotis diffusae*, *Phellodendron amurense*, *Sparganium stoloniferum*, Baicalin, and Baicalein, showed significant enhancement of radiation-induced growth inhibition as compared to radiation alone. Compared with radiation (3 Gy) alone, the enhanced growth inhibition rates for radiation in the presence of 5.0 µM Roscovitine, 12.5 µg/ml *Scutellaria baicalensis*, 25 µg/ml *Scutellaria baicalensis*, 500 µg/ml *Curcuma zedoaria*, 250 µg/ml *Hedyotis diffusae*, 500 µg/ml *Hedyotis diffusae*, 6 µg/ml *Phellodendron amurense*, 12.5 µg/ml *Phellodendron amurense*, 18 µg/ml *Phellodendron amurense*, 25 µg/ml *Phellodendron amurense*, 6.25 µg/ml Baicalin, 12.5 µg/ml baicalin, 3 µg/ml Baicalein, or 6.25 µg/ml Baicalein, were 26.8%, 19.5%, 59.3%, 32.9%, 41.5%, 47.4%, 27.7%, 54.5%, 57.0%, 74.9%, 49.0%, 71.8%, 32.0% and 55.4%, respectively (p<0.05).
Furthermore, the molecular basis for the enhanced growth inhibition of MDA-MB-231 breast cancers by a combination of radiation and herbal medicines was examined. The effects of radiation and herbal medicines on the cell cycle were examined by flow cytometric analysis. There was a significant increase in the accumulation of cells at the G2-M phase of the cell cycle after 5 Gy irradiation exposure. *Scutellaria baicalensis* and *Phellodendron amurense* combined with radiation (4 Gy) showed no significantly increase in Sub G1 and G2-M phase, compared with radiation alone. Moreover, radiation induced a 3~4 fold increase in micronuclei formation (a marker of chromosome damage) in MDA-MB-231 cells. *Scutellaria baicalensis* and *Phellodendron amurense* combined with radiation (4 Gy) showed no significantly increase in micronuclei formation, compared with radiation alone.

In conclusion, *Scutellaria baicalensis*, *Phellodendron amurense*, *Vaccaria pyramidata* showed significant radiosensitizing effect on MDA-MB-231 breast cancer cells, suggesting their potential for the development of novel radiosensitizers. Further study is needed to understand the underlying mechanisms.

Keywords: radiosensitizing effect, herbal medicines, human breast cancer cells, apoptosis, micronuclei