Introduction

Cancer therapeutics are roughly classified into two groups: (i) classical cytotoxic drugs that act on the cell division mechanism and suppress cell proliferation, and (ii) molecular-targeted drugs that target cancer-specific molecules that are essential for cancer growth and metastasis. The latter group of drugs is expected to show relatively lower side effects than classical cytotoxic drugs.

Based on these background, we investigated the cytotoxicity and hormesis-inducing activity of 12 classical and 8 molecular-targeted anticancer drugs, used for the treatment of cancer patients in our hospital, against 4 human OSCC cell lines (Ca9-22, SCC-25, HSC-5, HSC-8): 3 human normal oral mesenchymal cells (human gingival fibroblast (HGF), human periodontal ligament fibroblast (HPLF), human pulp cell (HPC)) and 2 human normal epithelial cells (human oral keratocyt(e)te (HOK), primary human gingival epithelial cells (HGEPI)). Since present treatment regimens of cancer patients usually adopt the combinations of more than two anticancer drugs, we also investigated whether such combination treatment induces synergistic cytotoxicity and apoptosis in HSC-2 cell line.

Materials and Methods

Cell culture: Human normal oral mesenchymal cells (HGF, HPLF and HPC) were established from the first premolar tooth extracted from the lower jaw of a 12-year-old girl, according to the guideline of intracellular ethical committee. Human oral squamous cell carcinoma (OSCC) cell lines (Ca9-22; derived from central tongue, HSC-5; HSC-8; derived from tongue) were purchased from Riken Cell Bank, Tsukuba, Japan. Human oral keratocyt(e)tes (HOK) (purchased from ScienCell Research Laboratories, Carlsbad, CA, USA) were cultured in oral keratinocyte medium. Primary human gingival epithelial cells (HGEPI) (purchased from CellLineTEC Advanced Systems AG, Bern, Switzerland) were grown in Culti-FR medium. HGF, HPLF and HPC at 15-20 population doubling levels (PDL), and HOK and HGEPI at 7-11 PDL were used in the present study.

Assay for cytotoxic activity: MTT method.

Calculation of tumor-specificity index (TS) = mean C50C against three normal oral cells (HGF + HPLF + HPC) / mean C50C against four OSCC cells (Ca9-22 + HSC-2 + HSC-5 + HSC-8).

Calculation of potency-selectivity expression (PSE) = 100 × TS / C50C (tumor cells) (100 × (HGF + HPLF + HPC) / (Ca9-22 + HSC-2 + HSC-5 + HSC-8) and 100 × C50C (HGF vs Ca9-22).

Calculation of combination index (CI): CI = (% decrease of viable cell number by compound A × % decrease of viable cell number by compound B) / % decrease of viable cell number by combination of compound A and B. The combination effect was judged “synergistic,” “additive” or “antagonistic” when CI < 1, 1 and > 1, respectively.

Cell-cycle analysis: Cell Sorter software version 2.1.2.

Classical anticancer drugs were more cytotoxic to OSCC than molecular-targeted drugs except bortezomib (Dose-response shift).

Weak hormetic effect (growth stimulation at lower concentration) of both classical and molecular-targeted drugs.

Anti-cancer activity of both classical (closed circle) and molecular-targeted drugs (open circle), defined as either TS or PSE, were correlated well with their cytotoxicity (C50C).

CDDP and 5-FU failed to produce subG0 population, but induced accumulation of G0/G1, and G0, phase cells, with or without combination treatment with C2C2 cells.

Most of anticancer drugs except CDDP and DDX (cited from interview form) were usually lower than the C50C for normal cells, indicating safety of the clinical administration.

Conclusion

The present study with a total 20 clinically used antitumor drugs demonstrates that classical antitumor showed higher cytotoxicity and antitumor potential as compared with molecular-targeted drugs except bortezomib.

When logarithm of antitumor potential of all compounds (either TS or PSE) was plotted as a function of cytotoxicity (C50C), highly significant correlation curves were produced. This suggests that anti-tumor potential of any unknown antitumor drug candidate can be easily estimated from this regression line.

Simultaneous addition of both types of anticancer drugs failed to induce apoptosis nor synergistic growth inhibition, suggesting the necessity of reconsidering the antitumor potency of present combination regimens with more than two compounds.

The present TS monitoring system may provide useful information for building up the treatment regimens of anticancer drugs.