THE THERMOSENSITIVITY OF HUMAN GINGIVAL SQUAMOUS CARCINOMA Ca9-22 CELLS WITH ONCOGENE erbB-1/EGFR

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The heat sensitivity of Human gingival squamous carcinoma Ca9-22 cells with oncogene erbB-1/EGFR and Chinese hamster V79 cells of normal thermosensitivity as control was investigated. Colony forming ability of the treated cells was assayed in vitro. Heat-treatment period-survival and the concerned curves were drawn. The slopes of exponentially regressing parts of the survival curves were estimated in the T₀ values of cellular thermosensitivity and subjected to Arrhenius analysis. The 42–44°C time-survival curves of Ca9-22 cells showed biphasic slopes which indicated the presence of thermotolerance induction during continuous heating even at 44°C while for the V79 cells the biphasic slopes due to thermotolerance induction were shown in temperatures at and before 42°C. In comparison of T₀ values with V79 cells, those of Ca9-22 cells were longer (less thermosensitive) by about 2.1 fold at 43°C and 1.2 fold at 44°C. Both V79 and Ca9-22 cells were sensitized by 44–42°C step-down heating (SDH). 42°C heat treatment period-survival curves of 44°C (5 minute) preheated V79 cells; while Ca9-22 cells under the same treatment condition for not only 5 minutes but 20 minutes showed biphasic slope, which indicated the presence of thermotolerance. The thermosensitization ratio (TSR) of Ca9-22 cells were smaller than V79 cells. Arrhenius curves a breaking points at 44°C and 43°C for Ca9-22 and V79 cells, respectively. The activation energies of V79 cells were 145 kcal/mole and 400 kcal/mole above and below 43°C (P < 0.05), respectively, while those of Ca9-22 cells were 200 kcal/mole and 250 kcal/mole above and below 44°C (P < 0.05), respectively. These data suggested that oncogene erbB-1/EGFR-contained in Ca9-22 cells may contribute to reduce thermosensitivity and variety in term of G₁ phase as shown in fractionated Hydroxyurea treatment.

Key words: Thermosensitivity, Oncogene.

Cancer is a multistep process resulting from the accumulation of genetic events effecting cell proliferation, involving oncogenes and tumour suppresser genes. A number of oncogenes have been identified in human tumour, e.g., H-ras in
bladder carcinoma, K-ras in human lung adenocarcinoma, C-myc in colon carcinoma, L-myc in small cell lung cancer, and erbB-1/EGFR in gingival squamous carcinoma.\textsuperscript{1-3} These oncogenes can be sort of growth factors, growth factor receptors, signal transducers or DNA-associated proteins. The constitutive activation of oncogenes has been shown to contribute directly to the malignant process and to the intrinsic susceptibility of human cancer cells to radiation, anticancer agents, or heat.\textsuperscript{4,5} In recent years, several lines of investigation have coalesced to demonstrate a link between certain oncogenes and the mechanisms underlying sensitivity or resistance to heating.\textsuperscript{6,7} Human gingival squamous carcinoma with amplified EGF receptor gene were adopted to determine its thermosensitivity and were compared with that in Chinese hamster V79 cells of normal thermosensitivity \textit{in vitro}. It is aimed to elucidate possible correlation of thermosensitivity and oncogene in human cancer cells.

\textbf{MATERIALS AND METHODS}

\textbf{Cells}

Human gingival squamous carcinoma Ca9-22 cells and Chinese hamster V79 cells were cloned and used for all the present experiments. Chinese hamster V79 cells have been long term cultured and Ca9-22 cells has not been cultured but mainly reserved under freezing.

\textbf{Culture Medium}

Ca9-22 cells were cultured for a term of the present experiment in a growth medium EM-10, composed of Eagle’s MEM solution (Nissui) supplemented with inactivated 10% Fetal bovine serum and antibiotics. V79 cells were maintained in a growth medium MLN-10, 1 liter of which were composed of 780 ml of Eagle’s MEM solution, 20 ml of 2.5% solution of lactalbumin hydrolysate (Difco), 100 ml of NCTC-135 solution (Difco), and 100 ml of inactivated bovine serum (Gibco).

\textbf{Hyperthermia}

Hyperthermia was carried out by immersion of culture flask in a temperature-regulated water bath (Toyo Seisakusho Co., Model EPS-47) preset at 39–45°C. The temperature was maintained within an error of \pm 0.05°C of the preset temperatures with a thermistor (Takara Thermistor Instruments Co., Model D116-1251).

\textbf{Experimental Procedures}

Exponentially growing cells in monolayers were trypsinized with 0.05% trypsin solution to obtain single cell suspensions in the medium. Cells were serially diluted with medium and seeded in appropriate cell numbers per flask (Nunclonn, 25 cm\textsuperscript{2}, 40 ml) with 6 ml medium to yield approximately 50 to 100 surviving colonies. The seeded cells in flasks with loosened screw tops were placed in a CO\textsubscript{2} incubator at 37°C overnight prior to the present series of experiments. The adhered cells on the inner surface of the flasks covered with 6 ml of medium were immersed in a water bath for treatment. (A) Single heating: Flasks of both Ca9-22 and V79 cells were immersed in a water bath for graded periods at temperatures 39°C to 45°C, respectively. (B) SDH-heating: Both Ca9-22 and V79 cells were preheated at 44°C for 5, 10, 20 minutes, respec-