

210 Heat sensitivity of DNA-dependent protein kinase activity

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Heat sensitivity of DNA dependent protein kinase (DNA-PK) activity was studied. DNA-PK activity is observed when DNA-PKcs (p460), Ku70, Ku80 and dsDNA fragment are associated. Scid cells lack DNA-PKcs but contain Ku70 and Ku80. Hybrid cells contain the fragment of human chromosome 8 with DNA-PKcs. DNA-PK activity of hybrid cells was inactivated by heat treatment at 44 °C. When the scid cell extract was added to heat-treated hybrid cell extract, the DNA-PK activity was recovered to notreatment level. This indicate that Ku70 and/or Ku80 are heat sensitive. Cell extract of sxi-3 cells that lack tKu80 could not recover the heat-inactivated DNA-PK activity. These results indicate that the heat sensitive component of DNA-PK activity was Ku80.

211 Heat resistance of a radiation-sensitive M10 mutant of L5178Y mouse lymphoma

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The molecular mechanism of radio- and thermoresponse has been investigated in the L5178Y mouse lymphoma cell line and its radiosensitive and thermoresistant M10 cells. By Western blotting protein assay we found out that in response to the heat treatment the heat-inducible heat-shock protein hsp72 was accumulated in both cell lines, where the magnitude of accumulation seems to be higher in M10 cells as compared with L5178Y cells. The constitutive hsc73 protein, in contrast, was detected only in M10 cells. Thus, the high inductability of the hsp72 protein and much amount of the hsc73 protein are likely to play a major role in the determination of thermoresistance of M10 cells. In addition, we suppose that L5178Y cells are negative cells in WAF1 induction by heat. This fact may partially explain the phenomenon that after heat treatment the L5178Y cells die by apoptosis without G1 arrest, as it was shown by FACScan analysis. Further investigation is needed, however, for the determination of the WAF1 gene status.

212 Appearance of Thermotolerance on TS Mutant AF-8 Cells

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PURPOSE To clarify mechanisms of thermotolerance, relationship between appearance of thermotolerance and exposure timing to non-permissive temperature of TS mutant AF-8 cells that were derived from BHK/21 cells was studied.

MATERIALS AND METHODS AF-8 cells were used in this experiment. Non-permissive temperature of this cells is 39.7°C. Cell culture was incubated at 34°C in CO₂ incubator. Heating was carried out with water bath. Cell survivals were determined by colony formation.

RESULTS D₁₀ of 43, 44 and 45°C of AF-8 cells were 135, 80 and 45 min, respectively. When AF-8 cells were kept at 34°C after first treatment of 45°C for 20 min, big magnitude of thermotolerance was observed. However, the tolerance disappeared completely, if cells were kept at 39.7°C after first heating of 45°C. Thermotolerance at 34°C reached to plateau 8 hrs after first heating at 45°C for 20 min.

When cells removed to 34°C from 39.7°C, the magnitude of thermotolerance became to smaller according the length of treatment time at 39.7°C