

Mutations in the Mitochondrial Genome of Pancreatic Cancer Confer Resistance to Anticancer Drugs

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The majority of cancer cells harbor homoplasmic somatic mutations in the mitochondrial genome. We show here that somatic mutations in mitochondrial DNA (mtDNA) are involved in the tolerance to anticancer drugs. We used trans-mitochondrial hybrid cells (cybrids) to reveal the role of mutations in mtDNA in pancreatic cancer by excluding any effects of the nuclear background. Cybrids were constructed by repopulating HeLa cells devoid of mtDNA with mtDNA derived from enucleated pancreatic cancer cell lines harboring mtDNA mutations (**Fig. 1**). This technique allowed the isolation of cybrid clones containing either homoplasmic mutants or homoplasmic wild-type mtDNA with a common nucleus derived from HeLa cells.

We used pancreas cancer cell lines CFPAC-1 and CAPAN-2 as donors of mtDNA containing mutations. These cell lines contain mtDNA mutations and are generally accepted. We constructed several cybrids with homoplasmic point mutations derived from the cancer cells as well as those with wild-type mtDNA derived from healthy individuals.

We compared the mutant and wild-type cybrids in resistance to an apoptosis-inducing reagent and anticancer drugs by exposing the cybrids to staurosporine, 5-fluorouridine, and cisplatin in vitro. The experiment clearly revealed that all the mutant cybrids were more resistant to the apoptosis-inducing drugs than were the wild-type cybrids. However, the resistance of cybrids to hyperthermia decreased.

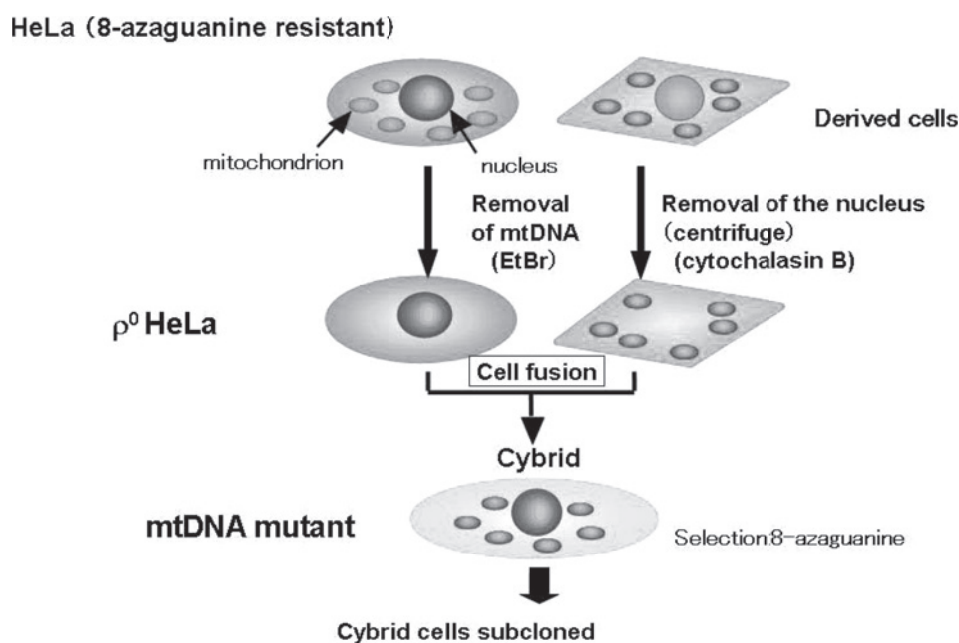


Fig. 1 Construction of cybrids

Next, the sub-G1 populations were examined to estimate nuclear DNA fragmentation during apoptosis. Treatment with staurosporine increased the sub-G1 populations to a greater extent in wild-type cybrids than in mutant cybrids. Notably, sodium azide, an inhibitor of cytochrome *c* oxidase, significantly prevented the increase in the sub-G1 population of wild-type cybrids with staurosporine, suggesting that respiratory chain activity is involved in staurosporine-induced apoptosis. Furthermore, we used immunostaining to investigate cytochrome *c* release from mitochondria. Most cells not treated with staurosporine showed high mitochondria membrane potentials. Most cells of wild-type cybrid lost $\Delta\Psi_m$ and were diffusely stained with an anti-cytochrome *c* antibody, indicating cytochrome *c* release to the cytosol. In contrast, mutant cybrids maintained their mitochondrial membrane potentials and the co-localization of cytochrome *c*. These results clearly indicate that mtDNA mutations of pancreatic cancer inhibit cytochrome *c*-dependent apoptosis.

Thus, we propose that mutations in the mitochondrial genome in pancreatic cancers are potential targets for anticancer drugs.
