101 RESTRICTION FRAGMENT LENGTH POLYMORPHISMS OF HPRT AND APRT GENES IN JAPANESE POPULATION

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HPRT and APRT are the enzymes of salvage pathway in purine metabolism. A virtural absence of HPRT activity is found in patient with Lesch-Nyhan syndrome and patients with APRT deficiency present 2,8-dihydroxy adenine urolithiasis.

A three-allele restricion fragment length polymorphism (RFLP) has been identified by Bam HI digestion at the human HPRT locus (Nussbaum et al PNAS, 80, 4035, 1983). DNA samples from 35 female and 68 male Japanese were analyzed and allele frequencies were compared with those in Caucasian. They are 0.45 for the 22kb/25kb allele, 0.34 for the 12kb/25kb allele, and 0.21 for the 22kb/18kb allele. Thus, the frequencies of the 12kb/25kb allele and the 22kb/18kb allele are apparently higher in Japanese population.

A two allele RFLP for Taq I at human APRT locus has been also identified (Stambrook et al, Somat. Cell Mol. Genet. 10, 359, 1984). The alleles are expressed as the fragment of 2.7kb or 2.1kb in size. DNAs of 72 unrelated Japanese individuals were analyzed; 20 individuals were homozygous for the 2.7kb allele, 18 individuals were homozygous for the 2.1kb allele, the remaining 34 individuals were heterozygotes. Thus, the frequencies of two alleles in Japanese population are significantly different from those in Caucasian, since the frequency of 2,7kb allele in the later population is 0.21. Bgl II digestion of DNAs from 114 unrelated individuals produced two different patterns. DNAs of 111 individuals produced a labeled fragment about 14kb in size. The 3 DNA samples produced an additional 17kb band, which was expected by the loss of a Bgl II site located upstream from APRT gene.

102 SYNERGISTIC INTERACTION BETWEEN ETOPOSIDE AND 1-β-D-ARABINOFURANOSYLCYTOSINE

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The sequence-dependency of the antitumor effect of etoposide (VP-16) and 1-B-D-arabinofuranosylcytosine (ara-C) and its mechanism was investigated in L1210 bearing BDF1 mouse. Treatment with VP-16 of 15mg/kg and ara-C of 25mg/kg was administered intraperitoneally on days 1,4,7 after tumor inoculation. Seven of 10 mice treated with three hour-pretreatment with VP-16 followed by ara-C were cured, but none of the mice treated with simultaneous administration was cured. Only 2 of 10 mice treated with the reverse sequence were cured.

To discuss the mechanism of this sequence-dependency, incorporation of ara-C into DNA was determined in combination with VP-16. On day 3 after tumor inoculation, VP-16 and 1μCi of (^{3}H)ara-C was injected intraperitoneally. Three hour pretreatment with VP-16 increased incorporation of ara-C up to 230% of ara-C injection alone, while simultaneous administration of VP-16 decreased it by 67%.

103 PURINE 5'-NUCLEOTIDASE--ITS REESTIMATED SUBUNIT MOLECULAR MASS AND IMMUNOCYTOCHEMICAL LOCALIZATION IN CHICKEN LIVER

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Purine 5'-nucleotidase, formerly termed cytosol 5'-nucleotidase (Tsushima, K. (1986) Adv Enzyme Regul 25, 181), is one of soluble nucleotidases including pyrimidine 5'-nucleotidase and deoxyribonucleotidase, and preferentially hydrolyzes IMP, GMP, and AMP in the presence of Mg^{++}. The enzyme has been investigated to have allosteric properties characterized by activation by ATP, ADP, 2,3-diphosphoglycerate, and diadenosine tetraphosphate. Purine 5'-nucleotidase has been purified from various sources, but structural studies seem to be incomplete. The subunit molecular mass of chicken liver enzyme, which was earlier reported to be 51 kDa upon SDS-PAGE, was reinvestigated. By immunoblot analyses after SDS-PAGE, a crude fraction from the liver homogenized in the presence of leupeptin showed multiple bands around 57 kDa, and SDS-extracted proteins directly from the liver exhibited a single immunoreactive 70-kDa band. In vitro translation products using chicken liver polysomes also showed a radioactive 70-kDa band after immunoprecipitation. Immunocytochemical study showed that the antigen was exclusively located in the cytoplasmic matrix of chicken liver sinusoidal and parenchymal cells, suggesting that physiological processing might not be the case with chicken liver enzyme. These results indicate that the subunit molecular mass of chicken liver purine 5'-nucleotidase might be 70 kDa, and the enzyme is the first case to be morphologically located in the cytosol among several known cytosolic nucleotidases.

104 A SCREENING METHOD FOR DIHYDROPYRIMIDINE DEHYDROGENASE DEFICIENCY WITH COLORIMETRIC DETECTION OF URINARY URACIL

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Dihydropyrimidine dehydrogenase (DHPDH) deficiency has a seizure as a common symptom among the reported cases. The other symptoms are mental retardation, hair abnormalities and so on. No major symptom exists for DHPDH deficiency. On the other hand, relationship between epilepsy and metabolic disorder is still obscure. For the purpose of finding more patients with DHPDH deficiency, we developed a screening method for DHPDH deficiency.

We applied a colorimetric determination method for urinary uracil detection. This method is not so complicated and less time consuming as previous methods such as thin layer chromatography. With this method, it is possible to detect at least 20 mg/dl of uracil, which is sensitive enough for the screening for DHPDH deficiency. Interfering substances in urine are negligible including drugs and foods. Addition of albumin to normal urine dose not affect the result but proteinuria results in false positive.

We screened urine from 83 epileptic children with this method, but could not find any patients.

105 THE SPECTRUM OF HPRT DEFICIENCY: AN UPDATE

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The severity of the symptoms accompanying HPRT deficiency were previously shown to be related to the amount of residual enzyme activity, as measured in intact cultured cells. We have now studied many more patients by this method and have a better idea of the degree of enzyme deficiency at which the various symptoms appear. We have also identified two new phenotypes of HPRT deficiency.

All the classic Lesch-Nyhan patients we have studied have had < 1.4% of the normal HPRT activity. These patients have uric acid overproduction, choreoathetosis, spasticity, mental retardation, and self-mutilative behavior. Their cultured cells are readily selected by 8-azaguanine. At the top of this range (1.4%) was a clinically classic Lesch-Nyhan patient whose cells are not selected by 8-azaguanine. Patients with >1.4 and <1.6% of the normal activity have had normal intelligence but otherwise all the Lesch-Nyhan symptoms. Patients with >1.6 and <8% of the normal HPRT activity have had neurological manifestations, but normal intelligence no self-mutilation. At the top of this range was a patient with no choreoathetosis, but with spasticity and uric acid overproduction. All of the patients we have studied who have had 8% or more (up to 60%) of the normal HPRT activity have had uric acid overproduction as their only clinical manifestation.

106 PURINE NUCLEOTIDE RESTORATION IN $HPRT^-$ CELLS

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Most metabolic therapies for HPRT deficiency have been aimed at restoring the nucleotide deficiency which is thought to occur as a result of the enzyme deficiency. None of these therapies has been successful, and this may be because they do not significantly increase purine nucleotides, particularly guanine nucleotides.

A number of precursors were tested for their ability to form purine nucleotides in both normal and $HPRT^-$ cells. In both cell types, adenine and adenosine were incorporated into purine nucleotides; virtually all this incorporation was into adenine nucleotides. Neither formamidoimidazole carboxamide (FAICA) nor its nucleoside (FAICAR) were incorporated. Aminoimidazole carboxamide (AICA) and its nucleoside (AICAR) formed purine nucleotides in both normal and $HPRT^-$ cells. AICAR was the more efficient nucleotide precursor in normal cells, whereas AICA was superior in $HPRT^-$ cells. These precursors produced approximately 90% adenine and 10% guanine nucleotides. Incorporation of AICA and AICAR was half maximal at approximately 200 uM and was not increased by addition of folate to the medium. Sodium formate greatly increased the incorporation of AICA and AICAR; this increase was half maximal at approximately 500 uM. Quantitatively, nucleotide production from 200 uM AICA or AICAR and 200 uM formate is greater than nucleotide production from a saturating concentration of hypoxanthine in normal cells. It is concluded that AICA or AICAR plus sodium formate could be used for nucleotide replacement in HPRT deficiency.