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BETA-GALACTOSIDASE DEFICIENT-TYPE MUCOLIPIDOSIS: A COMPLEMENTATION STUDY OF NEURAMINIDASE IN SOMATIC CELL HYBRIDS

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SUMMARY: By cell fusion with polyethylene glycol (PEG) a remarkable increase of neuraminidase activity was observed in the fused cells between ML-II and other two neuraminidase deficient disorders,ML-Gal(mucolipidosis, galactosidase deficient type) and ML-I(mucolipidosis I). No complementation was found in the combination of ML-I and ML-Gal. This result suggests that ML-I and ML-Gal may be allelic mutations and belong to the same genetic disorder as a primary neuraminidase deficiency.

INTRODUCTION

Beta-galactosidase deficient-type mucolipidosis (ML-Gal) is a neurodegenerative disorder with Hurler-like clinical features and neuraminidase deficiency. The characteristics of ML-Gal are very similar to those of mucolipidosis I (ML-I) except for the abnormality of β-galactosidase (1-3). ML-Gal has been frequently found among the Japanese (4), and now considered to be a single entity (1). But its pathogenesis remains unknown, and how this disease is related to ML-I has not been thoroughly clarified. This paper aims to present the cytogenetic and biochemical evidences to indicate that the etiology of neuraminidase deficiency of ML-Gal and ML-I is possibly common.

MATERIALS AND METHODS

Skin biopsies were obtained by the pinch technique and fibroblast culture was initiated and maintained in Eagle's minimum essential medium, supplemented with 10% fetal calf serum. The cell lines used in this experiment are summarized in Table 1. Patients with ML-Gal and Mucolipidosis II (ML-II) were diagnosed based upon the typical clinical and biochemical characteristics. Their skin fibroblasts were used for the previous complementation study (5). The cell line from the patient with ML-I was kindly provided by Dr.M.Cantz (Münster, West Germany).

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Table 1. Enzymatic characterization of the cell lines used in this experiment

Cell lines	β-Galactosidase		Neuraminidase
	Skin fibroblast	Medium	
ML-Gal	Deficient	Normal	Deficient
ML-I	Normal	Normal	Deficient
ML-II	Deficient	Excessive	Deficient

The induction of cell fusion with PEG between the cell lines with neuraminidase deficiency (Table 1) was performed by the method of Davidson and Gerald (6). Viable hybrid cells were grown to confluent, collected by trypsinization, washed three times with 0.15M NaCl, resuspended and homogenized in 0.3ml redistilled water with a Teflon-glass homogenizer.

Neuraminidase was measured towards fetuin, 2→3 neuraminlactose (2→3 NL), 2→6 neuraminlactose (2→6 NL), 2→3 neuramin[^{3}H]lactitol (2→3 [^{3}H]NL'ol) and 4-methylumbelliferyl N-acetylneuraminic acid (4MU-NANA). All assays were carried out in duplicate. The assay methods of neuraminidase towards fetuin and two NL's, and the sources of these substrates were described previously (1). 2→3 [^{3}H]NL'ol was prepared from 2→3 NL and the enzyme activity was measured according to Frisch et al.(7). For 4MU-NANA neuraminidase assay, 200μl of a incubation mixture which consisted of 1mM 4MU-NANA (kindly provided by Dr.J.S.O'Brien, UCSD,La Jolla, California, USA) and 70 to 100μg of protein of cultured skin fibroblast homogenate in 0.2M acetate buffer, pH 4.0, was incubated for 30min at 37°. Incubation was terminated by the addition of 0.2M glycine-carbonate buffer, pH 10.4, and the liberated 4MU was determined fluorometrically. Protein was measured by the method of Lowry et al.(8).

RESULTS AND DISCUSSION

In the cell lines derived from three different ML's (ML-Gal, ML-I and ML-II), the residual neuraminidase activities were low towards all sialic acid containing substrates used in this experiment (Table 2). Although the residual activity of 2→3 [^{3}H]NL'ol neuraminidase in all ML's was relatively higher than the other neuraminidase, these results are in good agreement with the data described previously on ML-Gal (1,3,7,9), ML-I (7,9,10) and ML-II (7,9,11).

The results of neuraminidase assays in the mixed cell and the heterokaryon cell population are shown in Table 3. Among three combinations, neuraminidase activities towards two different substrates in the fused cells of ML-Gal and ML-I were very close to the values in a mixture of parental cells (mixed cells) (0.9 nmol/mg protein/h in fused cells vs 1.o in mixed cells for 4MU-NANA; 28% vs 22% for [^{3}H]NL'ol), indicating that no complementation was observed between ML-Gal and ML-I. On the other hand, a remarkable increase

Table 2. Neuraminidase activities in cultured skin fibroblasts

Substrates	Normal Controls[3] (n=6)	ML-Gal	Parents of ML-Gal	ML-I	ML-II
Fetuin[1]	1.85 ± 0.41	0.08	0.89,0.97	0.01	0.03
2→3 NL[1]	13.3 ± 4.8	0.01	6.9, 8.0	0	0.03
2→6 NL[1]	5.3 ± 0.7	0	2.9, 2.6	0	0.02
4MU-NANA[1]	41.6 ± 2.1	1.1	---	0.45	1.8
2→3 [^{3}H]NL'ol[2]	103	27	---	21	23

[1] Enzyme activities are expressed as nmol of hydrolyzed substrate/mg protein/h.

[2] Enzyme activities are expressed as percentage of hydrolyzed substrate/mg protein/h.

[3] Mean ± 2S.D.

in neuraminidase activity towards both substrates was found when ML-II cells were fused with ML-Gal or ML-I. From these results it is clear that ML-II is different gene mutation from ML-Gal or ML-I. The restoration of β-galactosidase in the fused cells between ML-Gal and ML-II (5) supports this idea. At present both ML-Gal and ML-I are called sialidosis (3,12,13) because an abnormal urinary excretion of sialyloligosaccharides and a neuraminidase deficiency are demonstrated. In addition the clinical pictures of ML-Gal are similar to those of ML-I (1). From the clinical standpoint, Lowden and O'Brien (3) classified ML-Gal as juvenile type, and ML-I as infantile type in dysmorphic group of sialidosis. In our experiment restoration of neuramini-

Table 3. Neuraminidase activities in a mixture of parental cells (mixed cells) and fused cells by PEG in three combinations of different ML cell lines, and the occurrence of complementation.

Combinations of cell lines	4MU-NANA Neuraminidase (nmol/mg/h)	2→3 [^{3}H]Nl'ol Neuraminidase (%/mg/h)	Occurrence of complementation
ML-Gal x ML-II			
Mixed cells	1.3	18	
Fused cells	8.2	65	Yes
ML-Gal x ML-I			
Mixed cells	1.0	22	
Fused cells	0.9	28	No
ML-I x ML-II			
Mixed cells	1.6	18	
Fused cells	4.4	47	Yes
Normal Controls (n=6)	41.6	103	

dase activity was not detected in the fused cells between ML-Gal and ML-I. This result suggests that both ML-Gal and ML-I are allelic mutations and belong to the same genetic entity as a neuraminidase deficiency.

β-Galactosidase deficiency in ML-Gal is very unusual because it is limited in cultured skin fibroblast and peripheral leukocyte (1)(Table 1). This phenomenon seems to resemble the unusual distribution of hydrolases in ML-II. However, in the combination of ML-Gal and ML-II restoration of β-galactosidase (5) and neuraminidase occurred (Table 3). This indicates that the unusual enzymic distribution in both ML's is not due to the same defective process. The necessary factor(s) has been proposed to explain deficient β-galactosidase in ML-Gal (14), but further study on the modification of lysosomal enzyme moiety is necessary in order to understand this pathologic condition in ML-Gal.

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