Vol. 88, No. 2, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 28, 1979 Pages 559-562

THE COMPLEMENTATION OF β-GALACTOSIDASE IN FUSED CELLS OF MUCOLIPIDOSIS II WITH ANOTHER VARIANTS OF β-GALACTOSIDASE DEFICIENCY USING NEW SINGLE CELL ENZYME ASSAY

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Received February 20,1979

SUMMARY: By cell fusion and new single cell hydroalse assay technique, the complementation was observed between mucolipidosis II and other two hereditary lysosomal β-galactosidase deficient disorders, GM_1-gangliosidosis, type 2 and β-galactosidase deficient-type mucolipidosis. The possible mechanisms with which abnormal ML-II β-galactosidase was modified and normalized by other two different cell strains were discussed.

INTRODUCTION

Mucolipidosis II (ML-II, I-cell disease) is a rare hereditary disease. Cultured skin fibroblasts from the patients show deficiency in a set of lysosomal hydrolases including β-galactosidase(1). On the other hand, skin fibroblasts from GM_1-gangliosidosis, type 2 (GM_1-type2) (2) and β-galactosidase deficient-type mucolipidosis (ML-Gal) (3) have been known as cells showing deficiencies in β-galactosidase. ML-Gal is identical to GM_1-gangliosidosis, type 4 reported by Koster et al.(4). The present paper shows that the low β-galactosidase activity of ML-II cells is restored by fusion with ML-Gal or GM_1-type2 cells.

MATERIALS AND METHODS

Skin biopsies were obtained by the pinch technique and fibroblast culture were initiated and maintained in Eagle's minimum essential medium, supplemented with 10% fetal calf serum. Patients with ML-II, ML-Gal and GM_1-type2 were diagnosed by typical clinical and biochemical characteristics.

For the purpose of obtaining clear cut results on the complementation, a new micromethod to estimate lysosomal hydrolase activity of individual cells has been developed and reported elsewhere (5). In essence, a single cell (mono- and bi-nuclear cell) was spotted with phase contrast microscope after fused cells ($0.5\text{-}1 \times 10^3$ cells per 10cm Falcon dish) were seeded and incubated overnight. For incubation with 4-methylumbelliferyl-β-D-galactopyranoside (Koch-Light, U.K.) small plastic wells were settled on a dish. Fluorescence of librated 4-methylumbelliferone was measured manually with LDC Fluoro-Monitor after incubation was terminated by addition of 0.2M glycine-carbonate buffer.

0006-291X/79/100559-04$01.00/0

RESULTS AND DISCUSSION

Galjaard et al.(6) reported that ML-Gal and GM_1-type 1 or type 2 cells are complementary in β-galactosidase activity. Thus, the usefulness of our new method was tested using this known complementary combination. Figure A shows a distribution pattern of β-galactosidase activity of individual cells resulted after when a mixture of the two different cells was treated with UV-inactivated HVJ (Sendai virus). The pattern consisted of two groups of distribution; one population distributed in the range of original level of the two parent cells and the other distributed in the normal level which may be the population of the heterokaryon or hybrid of two kinds of parent cells.

Figure B and C show the distribution patterns of the enzyme level after the treatment of mixture of (ML-II and GM_1-type2 cells) or (ML-II and ML-Gal cells), respectively, with UV-HVJ. In both cases, the distribution patterns were very similar to the pattern of Figure A. A new cell population showing the normal level of β-galactosidase appears besides those of original low enzyme level. The former did not occur in homokaryocytes of the same cells after the treatment with HVJ. Moreover, K_m (0.33-0.37mM), pH optimum (4.4) and a gel filtration pattern through Sephacryl S-200 (Pharmacia, Sweden) of the newly occurred β-galactosidase by the cell fusion were identical to those of normal skin fibroblasts. These results suggest that ML-II cells are compremental with GM_1-type2 or ML-Gal cells in β-galactosidase activity.

The complementation between ML-II and the other two disorders can be explained by two theories. One is that the allelic correction occurred between both disorders. The other explanation, which seems more attractive, is that the correction occurred in the post-translational process of lysosomal enzymes in ML-II. It has been suggested that the pathogenesis of ML-II was the defective pinocytic mechanism of multiple lysosomal enzymes (1). Hickman et al.(7) proposed that carbohydrates were involved in the recognition marker on lysosomal enzymes. Several investigators (8-10) reported the evidences indicating that phosphorylated carbohydrate moiety was responsible for the recognition of

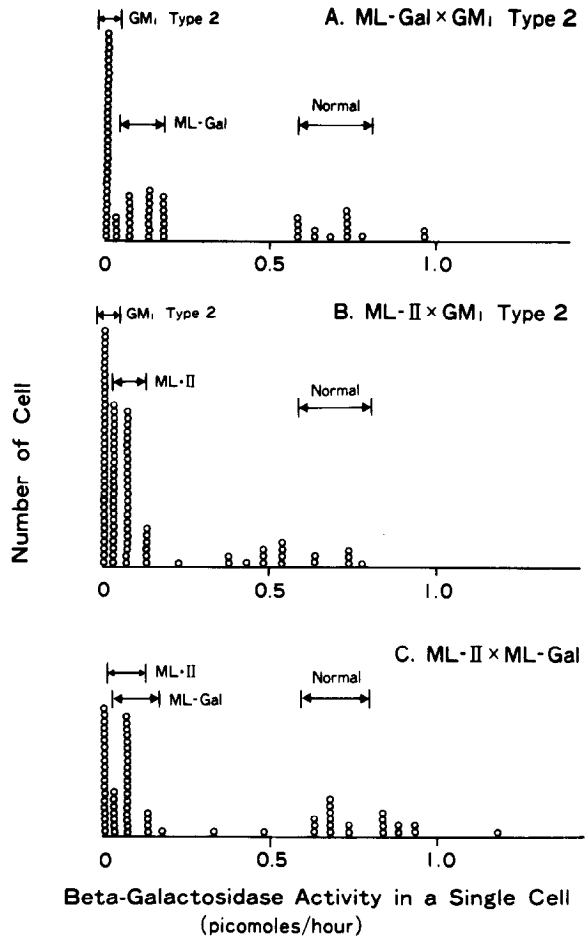

Fig. 1. Distribution of 4-methylumbelliferyl-β-galactosidase activity in a single cell after cell fusion with UV-inactivated HVJ (Sendai virus). The enzyme levels (Mean ± 2S.D.) in three different parental cells (ML-II, ML-Gal and GM_1-type2) and in normal cells are indicated in the Figure.

hydrolases by fibroblasts. Therefore it looks reasonable that in the heterokaryons ML-II lysosomal enzymes were phosphohexosylated correctly by golgi apparatus from the other disorder, and normal β-galactosidase moiety was produced. The phosphorylating system of lysosomal enzymes in golgi apparatus should be studied further to understand the primary defect of ML-II. However, another possibility still remains in the combination between ML-II and ML-Gal. The necessary factor (activator or aggregator) in ML-II which was originally

proposed in GM_1 by De Wit-Verbeek et al.(11) could also restore an incomplete enzyme in ML-Gal.

Correction of β-galactosidase defects in ML-II, ML-Gal and GM_1 offered the clear evidence indicating that these variants are based upon different gene mutations. And this complementation technique with single cell assay is very persuasive when the study of genetic variations is required. In addition, this simple method for single cell assay provides the tool for the study on heterozygous conditions in X-linked inherited disorders without cloning and the study on prenatal diagnosis with a very small number of cultivated cells.

ACKNOWLEDGEMENT

This study was supported in part by grants from the Ministry of Education and the Ministry of Health and Welfare in Japan.

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