SECRETION OF HUMAN ERYTHROPOETIN BY MAMMARY GLAND EXPLANTS FROM LACTATING TRANSGENIC RABBITS

Alina Aguirre, Alina Rodríguez, Boris Ramos, Pablo Fuentes, José de la Fuente and Fidel Ovidio Castro

Mammalian Cell Genetics Division.Center for Genetic Engineering and Biotechnology. P.O.Box 6162. Habana 6, Cuba.

Introduction

Production of foreign proteins in the milk of transgenic animals became a reality in the recent years. However, little is known about the regulation of transgenes in the mammary glands (MG) of transgenic animals. We studied the expression of a transgene and an endogenous milk gene in MG explants of lactating transgenic rabbits. Transgenic rabbits carrying the chimaeric gene: human erythropoetin cDNA (hEPO) under the control of a rabbit whey acidic protein promoter (rWAP) were generated.

Materials and Methods

Of a total of 795 microinjected embryos, 611 survived and were transferred to 44 recipient does, 43 founder animals were obtained. Among these pups, 6 were transgenics as judged by Dot blot assay, and 3 were females. One founder female, (F0-26) expressed low levels (0.3 ng/ml) of hEPO in the milk as detected by a commercial ELISA test (Boehringer Manhein, FRG). This female and her F1 transgenic progeny were mated. During lactation, small biopsies of thoraxic MG were aseptically taken on Days 10 and 15 for the FO-26, 7 and 14 for the F1 26-10 and non-transgenic female. After biopsy, mammary tissue was thoroughly washed by several passages in PBS+ 200 IU/ml of penicillin, 50 µg/ml gentamycin, and 0.2 mg/ml streptomycin.The tissue was cut in pieces of 2 to 3 mm, washed again in the same buffer and placed on a sheet of Kodak lens paper (1) in a 35 mm plastic Petri dish, floated with 2 ml of TCM-199 Earl's salts with a Hepes and bicarbonate buffer system, supplemented with antibiotics as described for PBS, and 5 µg/ml of insulin and prolactin and 2 µg/mL hydrocortizone. Explants were cultured for 5 to 7 d with daily change of medium, the supernatants were stored frozen at -70 °C, and at fixed time points the cultured explants were stored in liquid nitrogen for RNA analysis. For the ELISA, 50 µL of supernatant were used, while for the determination of rabbit WAP, proteins were precipitated with acetone, centifugated, the pellet resuspended in Laemli buffer and analyzed by SDS-PAGE and Western blot with a sheep heteroserum against rWAP, conjugated with horse radish peroxidase.

Results and Discussion

The hEPO was detected in supernatants from MG explants only of the FO-26 and her F1 progeny, in days equivalent to lactation Days 0 to 28. The expression levels were 0.185 ng/ml (range from 0 to 813.6) for the F0-26, and 0.122 ng/ml (range from 0 to 285.5) for the F1 26-10. Northen blot of RNA extracted from MG explants showed the presence of transcripts for hEPO and rWAP. Rabbit WAP was detected by Western blot, and its expression was present through all the days analyzed. The expression of hEPO in MG explants correlated well with the expression pattern of endogenous WAP gene during the entire lactation as shown by ELISA of the supernatants and RT-PCR from mammary gland biopsies of transgenic F1 rabbits. We can not rule out in our experiments, the possibility of ectopic expression of hEPO in non-epithelial tissues of the MG. This simple technique for culturing MG explants, could provide researchers with a tool to study gene expression in the mammary gland specially when the expression levels of the transgene are relatively low and therefore difficult to detect in entire milk.

1. Barash *et al.*, Transgenic Research 1993;2:266-276.