The SV40 T-antigen induces premature apoptotic mammary gland involution during late pregnancy in transgenic mice

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Abstract Transgenic animals of the line 8 contain the WAP-SV-T transgene. Females of this line synthesise the SV40 T-antigen in mammary gland epithelial cells during pregnancy and the lactation period. All females are 'milk-less' and the offspring have to be nursed by foster mothers. The reason for this phenomenon is a premature apoptosis during late pregnancy. Nonetheless a significant number of mammary epithelial cells escape apoptosis and all transgenic females develop breast cancer after the first lactation period.

Key words: SV40 T-antigen; Transgenic animal; Mammary gland; Premature apoptosis; Breast cancer

1. Introduction

During each pregnancy and lactation cycle, the mammary glands of rodents undergo an extensive differentiation process followed by gland involution after weaning. The glands of virgin animals contain exclusively adipose cells. During pregnancy, these cells are replaced by highly differentiated epithelial cells which form the alveolar and ductal structures that monopolise the gland during the lactation period. At this stage, mammary tissue accounts for about 20% of the body weight. Mammary gland development and milk protein synthesis (e.g. whey acidic protein) are under the control of various lactotrophic hormones (e.g. estrogen, progesterone, prolactin, insulin, hydrocortisone) and local growth factors. Shortly after lactation, the milk protein genes are switched off and the epithelial cells are again replaced by adipose cells [1–5]. Through this pre-programmed cell death (apoptosis) nearly 90% of the mammary mass is removed within a few days. The pre-programmed cell death seems to be a balanced cooperation between different genes that either promote or inhibit apoptosis [6]. We recently generated transgenic animals which carry the early SV40 coding region (T/t-antigen) under the transcriptional control of the whey acidic milk protein promoter (WAP-SV-T). Expression of the WAP-SV-T transgene occurs during pregnancy and the lactation period as demonstrated by immunofluorescence staining and Northern blot analysis [7]. After lactation, expression of the WAP-SV-T transgene continues in epithelial cells which escape mammary gland involution and as a consequence, all transgenic females develop breast cancer after the first pregnancy. Furthermore, all animals have a reduced lactation capacity and the offspring have to be nursed by foster mothers. The reason for this 'milk-less' phenomenon could be either an underdevelopment of the mammary glands during pregnancy or a premature mammary gland involution.

In this investigation we obtained experimental evidence that the SV40 T-antigen induces apoptosis during late pregnancy which causes involution of the mammary glands before birth of the litters.

2. Material and methods

2.1. Animals

Transgenic animals (strain: NMRI) carrying the WAP-SV-T transgene (WAP-promoter and the early SV40 *BglI*/*Bam*HI DNA fragment) were generated as described elsewhere [7]. The 1.6 kb WAP promoter was kindly provided by Dr. L. Henninghausen.

2.2. DNA fragmentation

DNA was extracted as described elsewhere [8]. In short, tissue segments were homogenised and then digested with Proteinase K (1 mg/ml, Boehringer Mannheim) in 1 ml of digestion buffer at 55°C for 20 hours. DNA (5 μg) was subjected to agarose gel electrophoresis (1.5% agarose gel 60 mA; running buffer: Tris-HCl 40 mM, Na-acetate 5 mM, EDTA 1 mM, pH 7.9). DNA fragmentation was visualised by ethidium bromide staining.

2.3. Histology

Mammary tissue segments were fixed in 10% formalin in phosphate-buffered saline (PBS) and defatted in acetone. After dehydration by means of graded concentrations of alcohol solutions, tissue segments were embedded in paraffin. Thin sections (4-6 μm) were stained with hematoxylin and eosin or Hoechst dye 33258 (8 μg/ml).

3. Results and discussion

Transgenic animals of the line 8 carry the WAP-SV-T transgene. This hybrid gene contains the early SV40 coding region (*BglI*/*Bam*HI DNA fragment) and the whey acidic protein-promoter. To test whether the 'milk-less' phenomenon of the transgenic animals is caused by an under-development of the mammary glands, tissue segments were isolated from normal and transgenic animals on the first day of the lactation period.

As shown in Fig. 1C, the under-development of the transgenic gland is evident. In this gland the adipose cells are the dominant cell type occupying about 40–50% of the entire gland tissue. In contrast, in the mammary gland of the normal animal few adipose cells are demonstrable and the epithelial cells are the dominant cell type, forming the lobuloalveolar structure of the gland (Fig. 1D). This observation implies that the SV40 T-antigen impairs the development of the mammary gland during pregnancy. To prove this histological sections were prepared from normal and transgenic animals on various days during pregnancy. We observed, however, that the mammary glands of the transgenic animals exhibited a higher degree of

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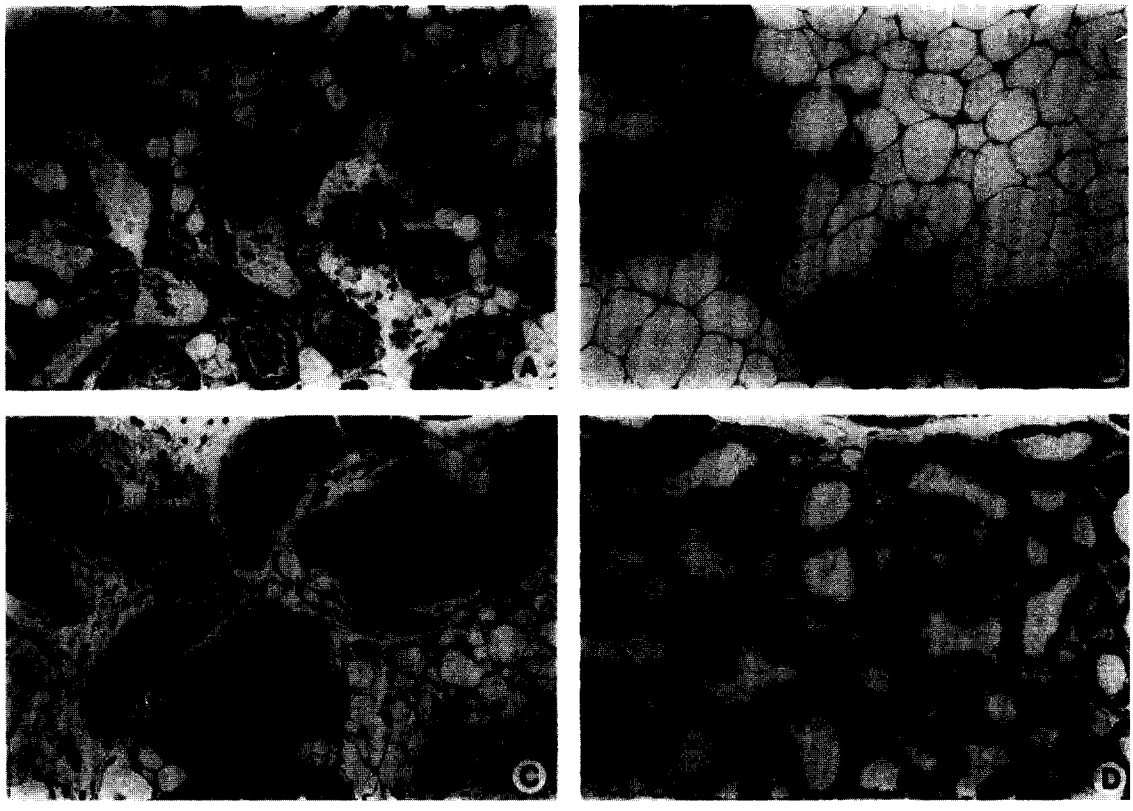

Fig. 1. Histology of mammary glands from transgenic and normal animals. Tissue segments were isolated: (A) from a transgenic animal at the day 18 of pregnancy; (B) from a normal animal at the day 18 of pregnancy; (C) from a transgenic animal at the first day of lactation; (D) from a normal animal at the first day of lactation.

development in late pregnancy than did those of the normal animals. As shown in Fig. 1A, on day 18 of pregnancy the mammary gland of the transgenic animals contained mainly epithelial cells while the gland of the normal animal still contained more adipose cells than epithelial cells (Fig. 1B). This observation indicates that the SV40 T-antigen stimulates epithelial cell proliferation rather than causing inhibition of cell division. This was not entirely unexpected since it is well documented that the SV40 T-antigen is a strong mitogen, inducing DNA replication and cell division in a wide spectrum of different cell types [9]. It is, therefore, more likely that T-antigen mediates a premature mammary gland involution during late pregnancy.

In normal animals, mammary gland involution takes place two to three days post-weaning, accompanied by a progressive decrease of WAP mRNA synthesis and replacement of the epithelial cells by adipocytes. About four to six days after weaning, the morphology and the expression rate of the milk genes (e.g. WAP, casein, lactalbumin) resemble the status of the mammary glands before pregnancy. The pre-programmed epithelial cell death, known as 'apoptosis' is characterised by distinct morphological alterations, including cytoplasm and chromatin condensation as well as nuclear segmentation. To obtain exper-

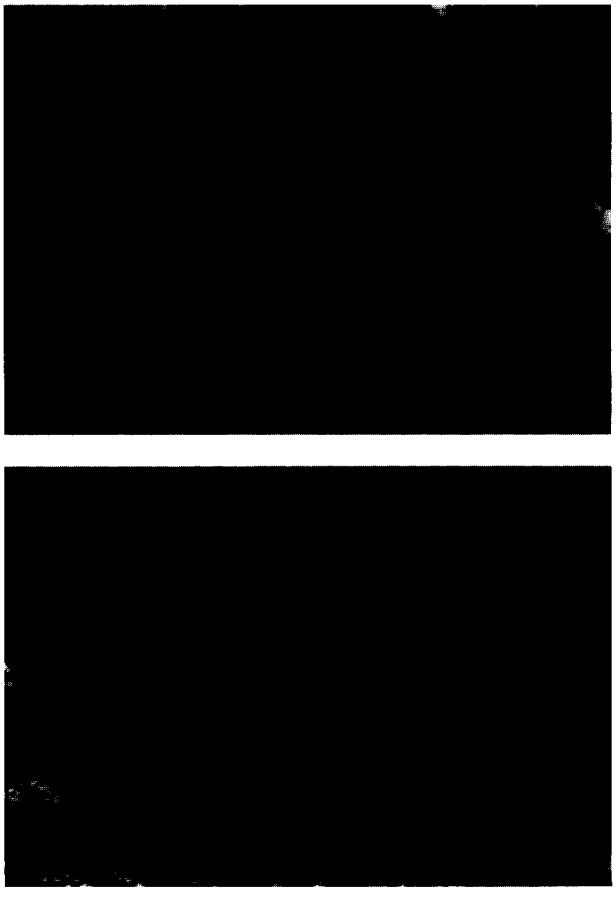

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Fig. 2. Mammary gland tissue sections isolated from transgenic and normal animals were stained with Hoechst dye 33258. Tissue segments were isolated: (A) from a transgenic animal at the day 18 of pregnancy; (B) from a normal animal at the first day of lactation.

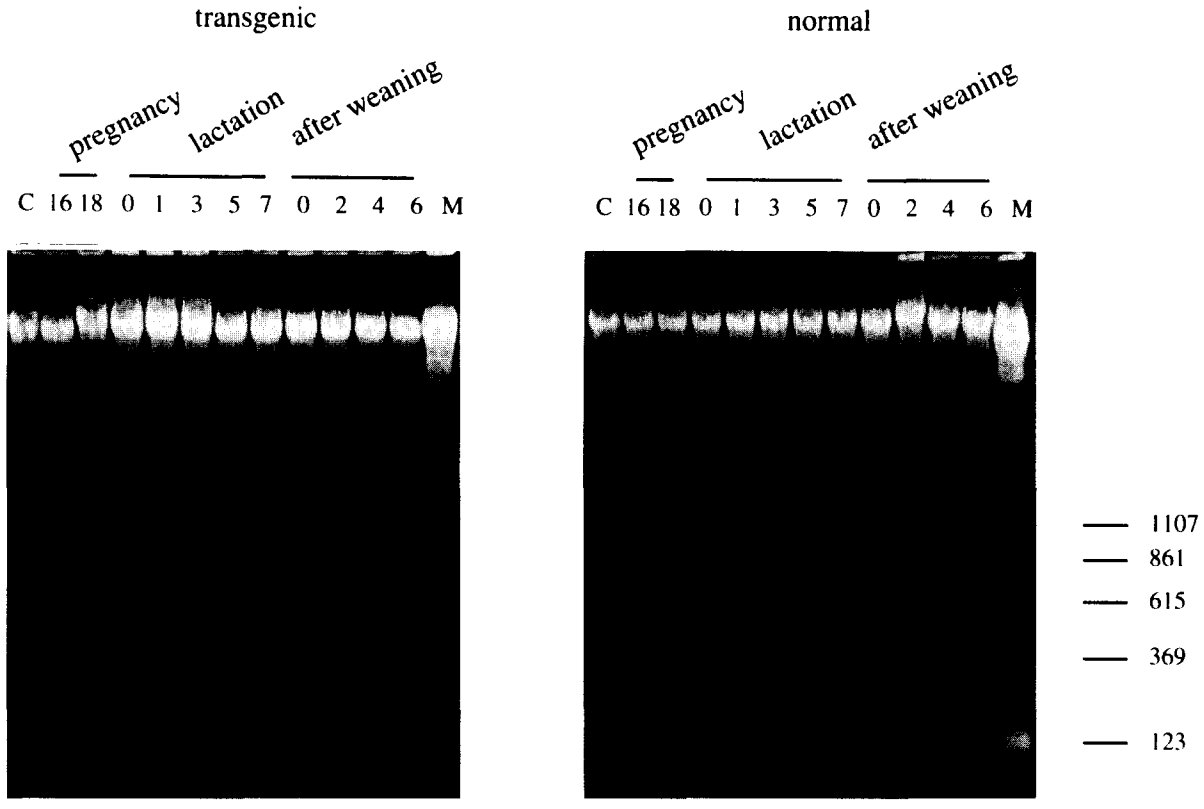

Fig. 3. DNA fragmentation in mammary gland cells of transgenic and normal animals: during pregnancy (day: 16 and 18), during lactation (day: 1, 3, 5, 7) and after weaning (day: 2, 4, 6). C: DNA was extracted from a tail segment of a transgenic animal. Tissue segments were isolated from the mammary glands at the time indicated; chromosomal DNA was extracted and subjected to agarose gel electrophoresis. DNA was visualised by ethidium bromide staining. The standard DNA fragments (M) are indicated on the right in bp.

imental evidence that the mammary gland involution of the transgenic animals during late pregnancy is caused by apoptosis, tissue sections were stained with Hoechst dye 33258. As shown in Fig. 2A, the mammary gland of the transgenic animals undergoes extensive apoptosis on the day 18 of pregnancy. This is indicated by clusters of abnormal epithelial cells with condensed nuclei, cytoplasmic condensation and nuclear fragmentation. Furthermore, large numbers of apoptotic cells are demonstrable in the alveolar and ductal lumen, suggesting that these apoptotic cells are cast-off from the alveoli. In contrast, mammary gland epithelial cells of the controls mainly exhibit a regular shape and apoptotic cells are almost not detectable in the alveolar and ductal lumen (Fig. 2B).

The biochemical hallmark of apoptosis is the non-random DNA fragmentation. This oligonucleosomal DNA fragmentation is specific for many forms of apoptosis including mammary gland involution [10–13]. To demonstrate further that premature cell death accounts for the under-development of the mammary glands at late pregnancy and during the lactation period, the DNA was extracted from normal and transgenic mammary glands and subjected to agarose gel electrophoresis. As shown in Fig. 3, DNA fragmentation is already demonstrable in the mammary glands of the transgenic animals on the day 18 of pregnancy and continues during the early period of lactation. In contrast, no DNA fragmentation is demonstrable in the mammary gland of the normal animals during pregnancy or the entire lactation period. DNA fragmentation occurs only two to four days after weaning, mediating the replacement of the epithelial cells by the adipose cells. These reults clearly demonstrate that the SV40 T-antigen acts as an intracellular inducer of apoptosis during late pregnancy, accounting at least in part for the milk-less phenomenon of the transgenic animals.

So far it is not clear how SV40 T-antigen causes premature apoptosis. However, this feature is common for several other viral proteins such as the papilloma virus 16 E7 and the adenovirus E1A protein [14]. These are mainly nuclear proteins with a strong mitotic activity. Recent studies indicate that apoptosis is regulated by different genes and that the cell death machinery is likely to be located in the cytoplasm [15–17]. Therefore, we have reasons to believe that the T-antigen-induced premature gland involution is an indirect effect requiring the interplay with other cellular proteins. This assumption is in accordance with the concept that apoptosis can be induced by activators of the cell cycle entry [6].

To test whether all epithelial cells are eliminated by apoptosis, tissue sections were prepared from transgenic animals during the lactation period and after weaning. T-antigen staining experiments revealed that mammary glands of the transgenic animals contained multiple islands of T-antigen-positive mammary epithelial cells (data not shown). This indicates that the SV40 T-antigen has a dual function: it mediates pre-mature apoptosis during late pregnancy and it allows a significant number of mammary gland epithelial cell to escape apoptosis. As a consequence of this escape, T-antigen-positive cells progressively divide and all transgenic females develop T-antigen-positive tumors after the first pregnancy [7,18].

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