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Differential Expression of DNA Repair Gene, N-Methylpurine-DNA Glycosylase during the Development of Balb/c Mice

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Balb/c 생쥐에서 DNA 회복효소인 N-Methylpurine-DNA Glycosylase(MPG)의 발생단계별 유전자 발현 조절

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요 약

DNA 회복효소인 MPG는 DNA의 퓨린기에 결합되어 있는 메틸기 등 이물질을 염기와 함께 제거하는 작용을 한다. 본 연구에서는 노던 블롯팅 방법을 이용하여 Balb/c mice의 각 조직별로 발생단계별 mRNA 발현 정도를 조사하였다. 뇌와 콩팥조직에서는 출생 직후에 발현이 가장 활발하였으며, 성체시기까지 비교적 높은 활성도가 유지되었다. 위장조직에서는 출생 직후에서 일주일 후까지는 명확히 관찰되었으나, 그 이후는 발현이 약화되었다. 간장과 폐조직에서는 그 발현 정도가 매우 약했으며, 특히, 간조직의 경우 출생 직후보다 성체에서 그 발현이 현저히 감소되었다. 이들 조직에서의 활성도는 출생후 24시간 이내에서 1주일후까지 상대적으로 높게 유지되다가 점차 감소되었다. 즉, 수유기(출생 직후부터 1주일후)에는 그 활성도가 성체시기(4주에서 6개월)보다 높게 유지되었다. 이러한 결과들로 미루어 보아 늙은 생쥐가 젊고 어린 생쥐보다 alkylating mutagen들에 노출되었을 때 암에 걸릴 위험성이 높다고 생각된다.

Key words: MPG, DNA repair, Ageing, Gene expression, Mouse, Tissue-specific, mRNA.

INTRODUCTION

DNA repair is a universal and ubiquitous process that is essential for cell survival. Lethal and mutagenic damages in DNA result not only from exposure to external chemical and physical agents but also from spontaneous chemical reactions, in particular, deamination of cytosine to uracil and spontaneous loss of purines. Such alterations usually block DNA replication, if left unrepaired would result in apurinic/apyrimidinic site. The alkyl adducts in DNA may lead to toxic and mutagenic responses (Mitra & Kaina, 1993). N-Methylpurine DNA glycosylase (MPG), a ubiquitous DNA repair enzyme, removes N-methylpurine and other lesion of purines induced in DNA. However, under special circumstances they can also lead to

mutations (Loeb & Preston, 1986). Such lesions could lead to dysfunction of cells and tissues, and they might well be the underlying cause of the age-related reduction of homeostatic capacity and the increased incidence of cancer and other diseases of old age. N-Alkylpurines in DNA may themselves be toxic to the cell (Karran *et al.*, 1982; Lindahl *et al.*, 1988). Even when the original lesions are quickly removed, they can still lead to secondary changes in the DNA, such as, in DNA-sequence and gene expression. This process would be accelerated when the efficiency of these molecular defense systems is declined in relation to age (Mullaart *et al.*, 1990).

There are substantial evidences on age-dependent loss of excision repair capacity in various mammals (Setlow, 1978; Kovacs *et al.*, 1984; Licastro & Walford, 1986; Washington *et al.*, 1989). There are also evidences for age-dependent decline in some DNA repair activities in healthy mammals (Kovacs *et al.*, 1984; Licastro & Walford, 1986). Gendler & Bernstein (1981) proposed that DNA damage may be an underlying cause for ageing when accumulating with age because of inefficient repair. The unrepaired lesions in the genome may cause various biochemical changes associated with normal ageing (Vijg & Knook, 1987). These results are consistent with the notion of a linkage between ageing and persistence of unrepaired damages in DNA (Wei *et al.*, 1993). They were able to detect an age-associated decline of 0.6%/year between ages 31 and 60 years in the DNA repair of human lymphocytes. According to Washington *et al.* (1989), the activity of mouse MPG is regulated in organ-specific and age-dependent fashion.

However, the molecular basis of regulation of MPG has not been extensively studied and also nothing is known about the tissue-specific and age-dependent transcriptional regulation of MPG. In order to study the possible age-dependence of repair of the alkyl adducts in DNA, we have measured the level of MPG mRNA in various organs at different developing stages. The present results showed that the MPG activity declines with age after suckling period.

MATERIALS AND METHODS

1. Preparation of mouse tissues

Mice were sacrified at newborn-stage, at 1, 2, 4 and 8 weeks and at 6-months (adult) after birth. The various organs (brain, kidney, liver, lung and stomach) were rapidly resected, dipped into liquid nitrogen and stored at −70℃ until use for RNA preparation.

2. Preparation of total cellular RNA and Northern blot hybridization

Total cellular RNA was isolated by the method of Chomzynski and Sacchi (1987). 20㎍ RNA in each lane of 1.2% agarose gel containing 2.2M formaldehyde was electrophoresed in 1 X MOPs buffer and transferred onto nylon membranes by capillary movement in 10 X standard saline citrate solution.

3. DNA probe preparation

The mouse MPG cDNA fragment (1.1kb) was prepared by EcoRI digestion of the pGI 1945 plasmid (Tatsuka *et al.*, 1995) and CHOb (0.74kb) fragment was obtained by the digestion of the

cDNA clone with XhoI and EcoRI which were derived from the rat cDNA library (Lim *et al.*, 1994). The fragments were radiolabeled using a [^{32}P] dCTP and used for Northern blot hybridizations.

RESULTS

Fig. 1 shows the tissue specific mRNA level of MPG in the newborn to mature adult animals in Balb/c mice. MPG expression in brain and kidney was very active within 24h after birth and the level was maintain after 6-month-old adults. MPG expression in stomach was also clearly detected from newborn to 1-week-old after birth and then the levels become very weak. MPG was barely expressed in the liver and lung, and it was very much reduced in the adult liver as compared to that in the newborn mice. Decreased expression of MPG in brain, kidney, liver, lung and stomach was detected (Fig. 2). The MPG mRNA levels were consistently higher in suckling (newborn and 1-week-old) mice than in young and mature adults. MPG mRNA level in stomach was relatively stable until adult, but, the expression is not the highest mRNA level. The liver has a relatively low level of MPG expression, and in most instances brain and kidney have a higher mRNA level than liver (Washington *et al.*, 1988, 1989).

MPG mRNA level in suckling mice (newborn and 1-week-old) was higher than young (4- and 8-week-old) and mature adults (6-month-old) in all organs tested. MPG expression was higher during suckling period than in young and mature adults.

DISCUSSION

In order to understand the tissue-specific and

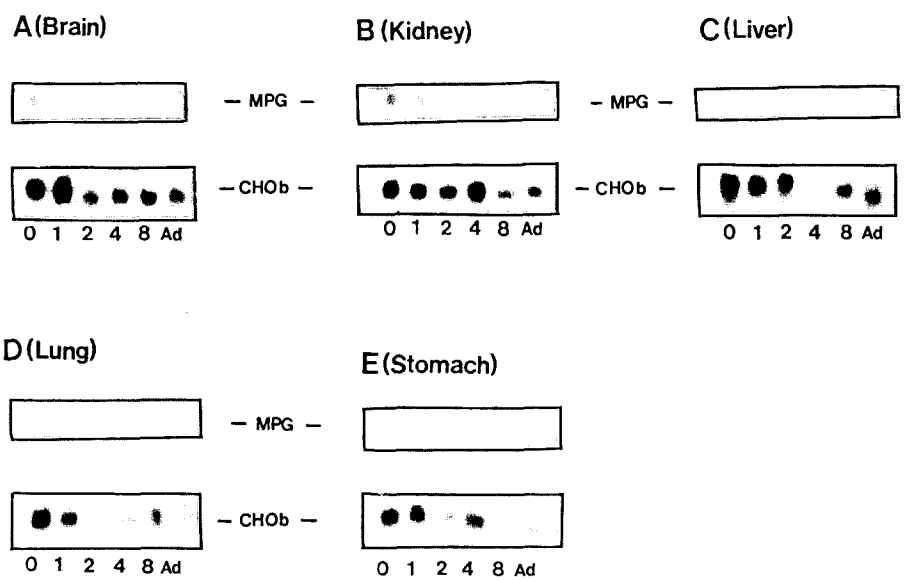

Fig. 1. Differential expression of MPG in the brain, kidney, liver, lung, stomach.
Total cellular RNA purified from the newborn mice (0), 1 week (1), 2 week (2), 4 week (4) and 8 week (8) and 6 month (Ad) old mice were separated on the agarose gel and the level of expressions was determined.

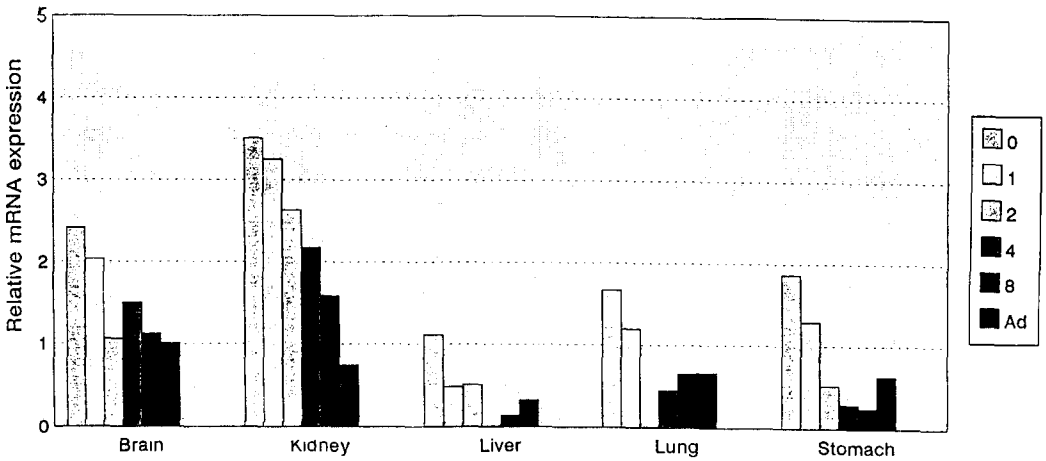

Fig. 2. Age-dependent levels of MPG mRNA in the tissues of Balb/c mice.
The level of MPG expressions was calculated using laser densitometry.

age-dependent regulation of MPG gene at the transcriptional level, we have analyzed changes of MPG expression in mice from newborn through 6-month-old adults. There were significant tissue- and age-specific differences in the mRNA level of MPG. Expression of MPG in brain, kidney, liver, lung, and stomach in mice from newborn through 1-week-old after birth was stronger than that of the 4-, 8-week and 6-month-old mice (Fig. 1). This trend was uniformly observed in the 5 organs. This might be implicated in a systemic loss of MPG expression in young and mature adult mice. MPG expression in brain, kidney was relatively stable in mice of different developmental stages including newborn through 6-month-old adults (Fig. 1). MPG expression was more active in suckling period than young and mature adults. These results suggest that the older mice are at a higher risk than younger mice following exposure to alkylating mutagens.

However, these patterns were different from the results of Washington *et al.* (1989). They reported that MPG activity is increased from birth to young adult stage in liver, lung, brain and ovaries of female mice of two inbred stocks, C3Hf and C57BL/E. The MPG activity was lower both in suckling animals (9-day-old) and in mature adults (17-month-old) than in young adults (7- or 8-week-old) in all four organs tested, namely, liver, lung, brain and ovary in both strains of mice. It appeared that the MPG activity level went up with age starting after birth and reached the maximum at the young adult stage. It was interesting to observe that the MPG activity level was lower both in suckling and in mature adult than in young adults of C3Hf and C57BL/E strains. And, the level of MPG expression in mature adult was the lowest. On the contrary, in our study, MPG mRNA level in brain, kidney, liver, lung, and stomach was significantly lower in young and mature adults than in suckling mice. However, our results based on mRNA level cannot be compared to their results based on enzyme activity of MPG in cell-free extracts.

Many investigators have proposed a linkage between aging in mammals and accumulation of lesions that could be induced either by exposure to environmental agents or from spontaneous endogenous chemical reaction. N-methylpurines in general, and 7-methylguanine in particular, have been implicated in the ageing process on the basis of circumstantial evidence (Gensler & Bernstein, 1981). Excision repair in response to exposure to alkylating agents is significantly decreased in old animals (Bond & Singh, 1987). At the same time, 7-methylguanine appears to accumulate in the DNA of aging mice. Endogenous damage to DNA may be a major contributor to both aging and cancer (Park and Ames, 1988). However, we cannot conclude from these results alone that N-alkylpurines will accumulate in the DNA of older animals without exposure to alkylating agents, because even the reduced glycosylase MPG activity may be more than enough to remove the small amount of N-methylpurines (e.g. 7-methylguanine) spontaneously induced (Mitra & Kaina, 1993). This may reflect a general loss of physiological efficiency in old animals and the possibility of age-dependent regulation of expression of specific genes could not be excluded. A comprehensive survey of repair efficiency for various type of DNA lesions is needed for elucidating the possible role of DNA repair loss in ageing (Washington *et al.*, 1989).

There are two characteristic features of organogenesis that are relevant to these studies. One is the altered cellular make-up of some organs, such as liver in particular, as a function of age. Hematopoietic cells make up a large fraction of the liver in young animals and the hepatocytes in older animals are often polyploid. Thus, the modulation of repair gene expression levels may reflect changing cell types and overestimate the enzyme content per cell when expressed as activity per μg DNA (Washington *et al.*, 1989).

Finally, it is not known whether the age-dependent changes in MPG activities are a reflection of the transcription rates of their genes, or result from altered stability of their mRNAs and their translation. Also, the significance of the present results in regard to the change in level of alkylation repair in ageing is not clear. Such molecular studies were not possible until the recent success in the cloning of the alkylation repair genes (Engelward *et al.*, 1993; Tatsuka *et al.*, 1995). Nevertheless, it appears reasonable that a systematic study should be undertaken to determine the level of alkyl adducts in several organs of a test animal as a function of age following a chronic low level of exposure to alkylating agents (Washington *et al.*, 1989; Mitra *et al.*, 1992; Mitra & Kaina, 1993).

Our study did not include old (1 or 2 years) animals. Therefore, we cannot conclude whether the MPG mRNA level continues to decline with age. It is interesting to note that N-methylpurines are known to be toxic to the cell and the loss of MPG in older adults may result in inefficient removal of these adducts which, in turn, may lead to toxic response and cellular death. These data indicate the decreased expression of the MPG as a possible marker gene of the aging.

ABSTRACT

N-Methylpurine-DNA glycosylase (MPG), a ubi-

quitous DNA repair enzyme, removes N-methylpurine and other lesion of purines induced in DNA.

The mRNA levels of MPG were investigated in various organs of Balb/c mice at different growth periods after birth by Northern blot hybridization. MPG expression in brain and kidney was very active within 24h after birth and the level was maintained until 6-month-old adults. MPG expression in stomach was also clearly detected in newborn as well as 1-week-old mice and then the levels become weak. MPG was barely expressed in liver and lung, and it was very much reduced in the adult liver as compared to that in the newborn mice. MPG expression in the organs was relatively active within 24 h and 1-week-old after birth and then the level becomes decreased. The suckling animals (newborn to 1-week-old) have consistently higher MPG mRNA level than young (4- and 8-week-old) and mature (6-month-old) adults. These results suggest that the older mice are at a higher risk than younger mice following exposure to alkylating mutagens.

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