表4.妥当性検討の結果(続き)
(High riskを5年罹患確率≥0.5%とする)

候補	ログランク検定	5y罹患確率(%)	E/O	感度/特異度(%)
3.Cox	P<0.0001	H=0.6 / L=0.3	1.72(1.12-2.68)	22/82
3.AF EXP	P=0.0001	H=0.4 / L=0.2	1.54(1.12-2.04)	54/60
3.AF Weibull	P=0.0135	H=0.3 / L=0.2	1.38(1.11-1.71)	96/9
3'.Cox	P=0.0082	H=0.5 / L=0.3	1.65(1.12-2.45)	28/82
3'.AF EXP	P=0.0027	H=0.3 / L=0.3	1.94(1.42-2.64)	51/60
3'.AF Weibull	P=0.1008	H=0.3 / L=0.3	1.40(1.12-1.73)	96/7
4.Cox	P<0.0001	H=0.7 / L=0. 3	1.40(0.91-2.14)	24/90
4.AF EXP	p=0.0002	H=0.5 / L=0. 2	1.20(0.85-1.69)	32/79
4.AF Weibull	.	H=0.3 / L=.	1.31(1.07-1.62)	100/0

表5.妥当性検討の結果
(High riskを5年罹患確率≥1.0%とする)

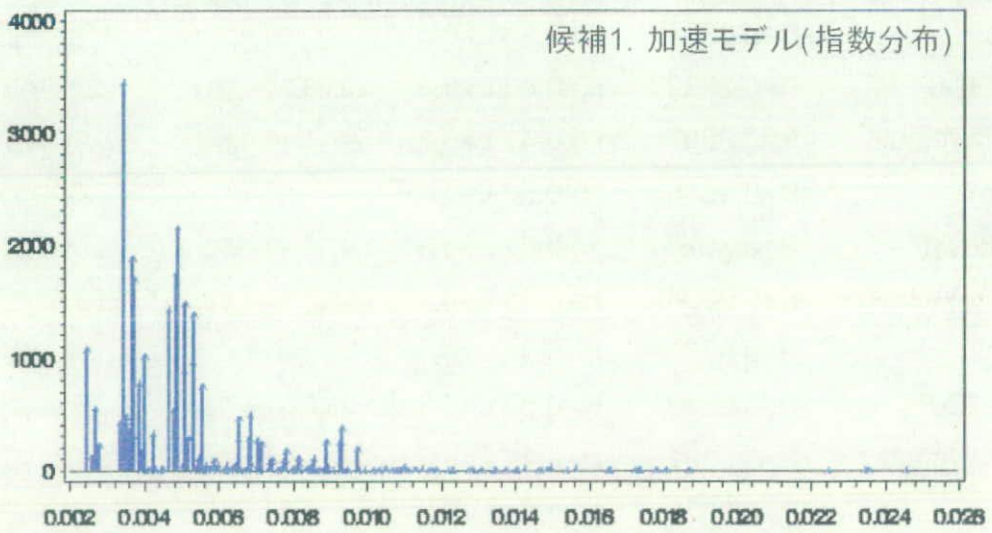
候補	ログランク検定	5y罹患確率	E/O	感度/特異度(%)
1.Cox	P=0.0854	H=0.9 / L=0.3	0	1% / 100%
1.AF EXP	P=0.2232	H=0.8 / L=0.3	1.23(0.31-4.91)	2%/99%
1.AF Weibull	P=0.0007	H=0.4 / L=0.2	1.47(1.14-1.88)	69%/45%
1'.Cox	P=0.4648	H=0.5 / L=0.3	0	1%/99%
1'.AF EXP	P=0.3075	H=0.6 / L=0.3	2.97(1.12-7.92)	5%/98%
1'.AF Weibull	P=0.0004	H=0.4 / L=0.2	1.45(1.13-1.87)	70%/45%
2.Cox	P=0.0981	H=0.9 / L=0.3	0	1%/100%
2.AF EXP	P=0.2232	H=0.8 / L=0.3	1.23(0.31-4.91)	2%/99%
2.AF Weibull	P=0.0007	H=0.4 / L=0.2	1.47(1.14-1.88)	69%/45%
2'.Cox	P=0.0322	H=1.3 / L=0.3	0	2%/99%
2'.AF EXP	P=0.0342	H=0.8 / L=0.3	1.91(0.72-5.08)	5%/98%
2'.AF Weibull	P=0.0114	H=0.4 / H=0.3	1.57(1.18-2.08)	55%/54%

表5.妥当性検討の結果(続き)
(High riskを5年罹患確率≥1.0%とする)

候補	ログランク検定	5y罹患確率(%)	E/O	感度/特異度(%)
3.Cox	P=0.0830	H=0.9 / L=0.3	0	1%/100%
3.AF EXP	P=0.2407	H=0.7 / L=0.3	0	1%/100%
3.AF Weibull	P=0.0001	H=0.4 / L=0.2	1.54(1.17-2.04)	55%/60%
3'.Cox	P=0.1783	H=0.8 / L=0.3	0	1%/100%
3'.AF EXP	P=0.1087	H=0.6 / L=0.3	0.65(0.21-2.00)	3%/98%
3'.AF Weibull	P=0.0006	H=0.3 / L=0.3	1.76(1.33-2.34)	55%/53%
4.Cox	P=0.4895	H=. / L=0.3	.	0%/100%
4.AF EXP	P=0.4895	H=. / L=0.3	.	0%/100%
4.AF Weibull	P=0.0002	H=0.5 / L=0.2	1.20(0.85-1.69)	36%/79%

図4. 予測5年罹患確率と人数

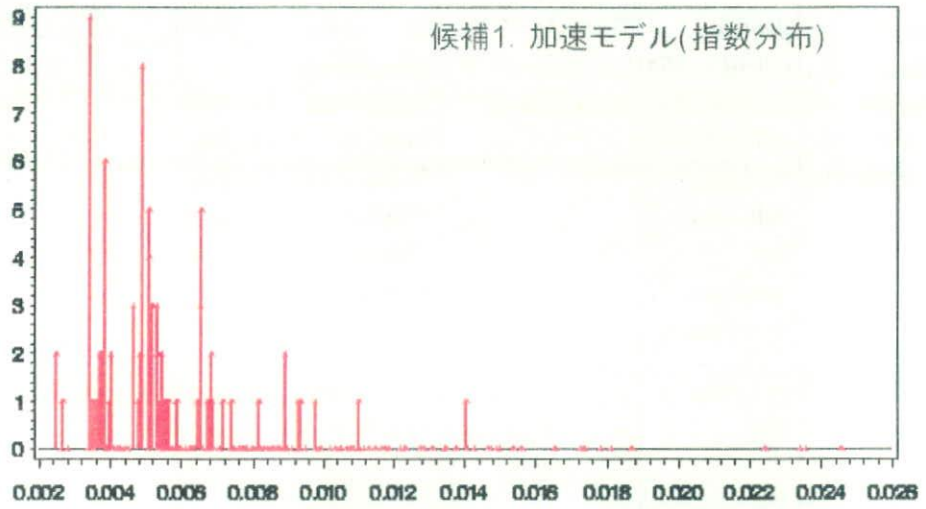
各リスクパターンに属する人数(コホートⅡデータ)



各リスクパターン(160通り)の予測5年罹患率

図5. 予測5年罹患確率と罹患者数

5年以内罹患者数(コホートⅡ データ)

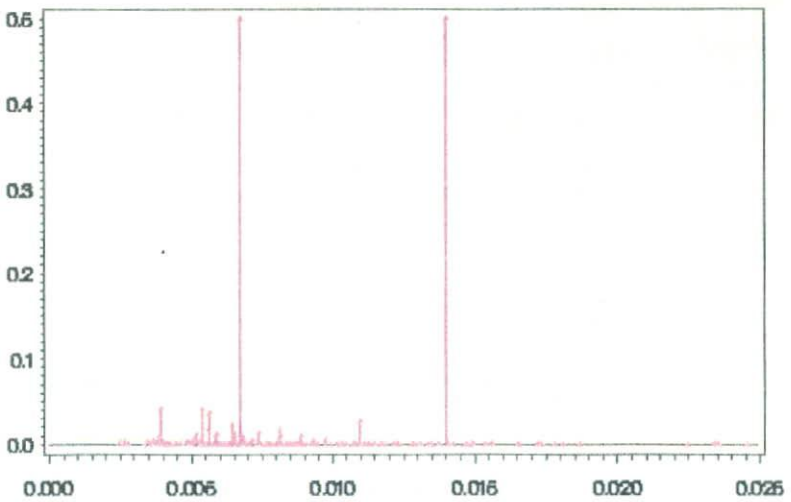


各リスクパターン(160通り)の予測5年罹患率

図6. 予測5年罹患確率と罹患割合

5年以内罹患割合(コホートⅡ データ)

候補1. 加速モデル(指数分布)



各リスクパターン(160通り)の予測5年罹患率

研究成果の刊行に関する一覧表

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Combined Effect of Ionizing Radiation and *N*-Ethyl-*N*-Nitrosourea on Mutation Induction and Lymphoma Development

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Summary. Carcinogenesis in humans is thought to result from exposure to numerous environmental factors. Little is known, however, about how these different factors work in combination to cause cancer. Mouse thymic lymphoma is a good model for research on radiation and chemical carcinogenesis. We examined here the occurrence of thymic lymphoma and mutation induction following exposure to both X-rays and *N*-ethyl-*N*-nitrosourea (ENU) in B6C3F1 mice. Mice were exposed weekly to whole-body X-irradiation (0.2 or 1.0 Gy per each exposure) for 4 consecutive weeks, ENU (200 ppm) in the drinking water for 4 weeks, or X-irradiation followed by ENU treatment. The incidence of lymphoma after 0.2 and 1.0 Gy were 0% and 10%, respectively. ENU treatment induced lymphoma in 20% of exposed mice. When ENU was combined with 1.0 Gy, lymphoma incidence increased up to 94%, showing a synergistic effect. In contrast, combination of ENU with 0.2 Gy resulted in a decrease in lymphoma incidence, that is, an antagonistic effect. Mutant frequency of the reporter transgene *gpt* after ENU exposure alone increased by tenfold compared to untreated controls. Combined exposure of ENU with 0.2 Gy X-rays dramatically decreased mutant frequency. In contrast, 1.0 Gy X-rays combined with ENU further enhanced mutant frequency and accelerated clonal expansion of mutated cells. In conclusion, the mutagenic and carcinogenic effect of combined exposure of X-rays with ENU is dose dependent.

Key words Thymic lymphoma · Combined genotoxic effect · *N*-Ethyl-*N*-nitrosourea · Radiation · Clonal expansion

Introduction

Human beings are exposed to numerous natural and man-made agents that are potentially carcinogenic. Therefore, cancer risk by ionizing radiation should be assessed as a result of combined exposures with other agents, including tobacco,

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genotoxic and nongenotoxic chemicals, hormones, viruses, and metals. Alkylating agents are found in plants, food, cigarette smoke, fuel combustion products, and commonly used industrial solvents. Some alkylating agents are also used for cancer therapy in combination with ionizing radiation. However, available data on the combined effect are relatively few, especially on its mechanism. In the present study, we report the dose dependence of the mode of combined effect of radiation with *N*-ethyl-*N*-nitrosourea on mutation induction and lymphoma development.

Materials and Methods

Female B6C3F1 mice were exposed to whole-body X-irradiation at a weekly dose of 0.2 or 1.0 Gy for 4 consecutive weeks or to *N*-ethyl-*N*-nitrosourea (ENU) at 200 ppm in drinking water. The mice were also exposed to X-rays followed by ENU (Fig. 1). X-ray irradiation was performed at a dose rate of 0.7 Gy/min. The mice, which had symptoms of thymic lymphoma 2 to 4 months after exposure, were killed under anesthesia and autopsied. The B6C3F1 *gpt*-delta mice were similarly treated with both X-rays and ENU. After the 4th week of exposure, the thymus was analyzed for the frequency and spectrum of *gpt* mutation as described previously [1]. Recurrent mutations derived from the tissue of a single animal could be the result of clonal expansion that occurred early after mutagen treatment.

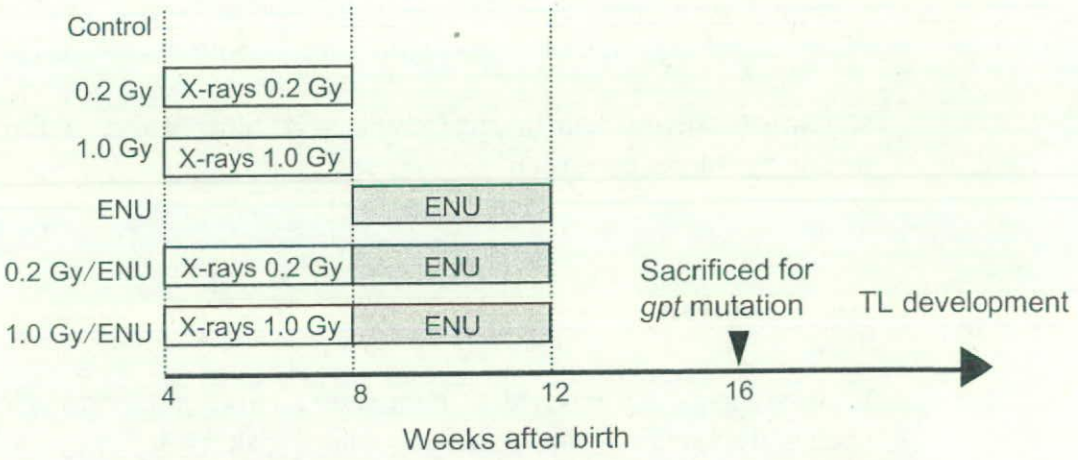

Fig. 1. Experimental design for thymic lymphomagenesis (TL) and *gpt* mutation analysis in mice treated with X-ray irradiation, *N*-ethyl-*N*-nitrosourea (ENU), or a combination of the two. Mice were exposed to X-rays weekly. ENU was administered at a concentration of 200 ppm in drinking water

Results

Combined Effect of X-Rays and ENU on Thymic Lymphomagenesis

Repeated exposure to X-rays at 1.0 Gy per exposure (4.0 Gy in total) increased the incidence of thymic lymphomas to 10%, whereas X-rays at 0.2 Gy did not induce lymphomas at all. ENU at 200 ppm induced lymphomas at an incidence of 20%. Combined exposure of ENU with 1.0 Gy X-rays resulted in a dramatic increase in lymphoma incidence at 94%, indicating a synergy. When ENU was combined with low-dose X-rays (0.2 Gy), the incidence was significantly reduced compared to that of ENU treatment alone, suggesting a protective role of low-dose X-rays for ENU-induced lymphomagenesis.

Induction of Gpt Mutation after Combined Exposure

DNA mutations play a central role in carcinogenesis. The frequency and type of mutations that result from combined treatment may shed light on the molecular mechanism(s) underlying the carcinogenic effects of combined exposure to ENU and radiation. To delineate such mechanisms, we have examined the occurrence of mutations in thymic cells of B6C3F1 (*gpt*+/–) mice after combined exposure [2]. It was found that ENU increased mutant frequency by ten-fold relative to untreated controls (Fig. 2). The mutant frequency in mice exposed to 0.2 Gy or 1.0 Gy X-rays alone was, surprisingly, reduced compared to the control ($P < 0.05$). Exposure to high-dose X-rays (1.0 Gy) followed by ENU increased mutant frequency by three-fold relative to ENU alone and facilitated clonal expansion of mutated cells. When low-dose X-ray (0.2 Gy) was combined with ENU, mutant frequency was, unexpectedly, reduced, which was primarily the result of a decrease in G:C to A:T and

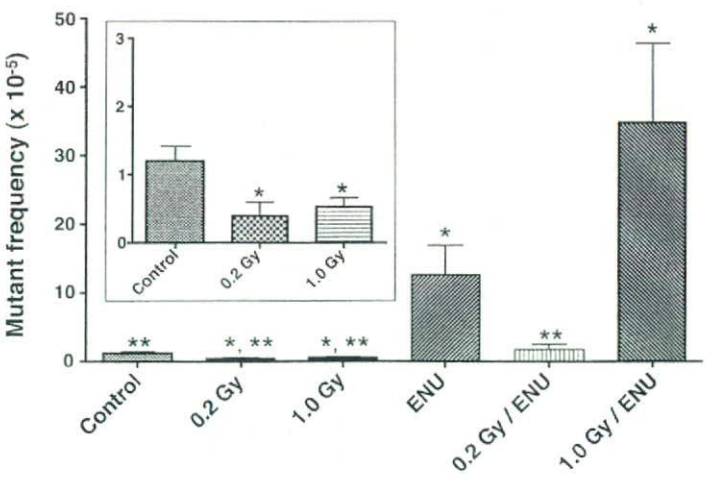

Fig. 2. Mutant frequency analysis of *gpt* recovered from thymus DNA from control, irradiated (0.2 Gy or 1.0 Gy), ENU-treated, and irradiated/ENU-treated mice. The *inset* shows an expanded scale for mutant frequency for the first three conditions. $*P < 0.05$, significantly different from control; $**P < 0.05$, significantly different from ENU. *Bars* represent mean ± SD

A:T to T:A mutations. In addition, clonality was drastically reduced compared with ENU alone (24.6% vs. 82.2%). The mode and mechanism of combined exposure clearly differs between low and high doses of radiation.

Discussion

We report here the dose dependency of the mode of thymic lymphomagenesis and mutagenesis after combined exposure to X-rays and ENU. It was shown that low-dose X-rays suppressed lymphoma induction by ENU whereas high-dose X-rays enhanced induction. In accord with this, low-dose X-rays reduced ENU-induced mutant frequency and clonal expansion of mutated cells and high-dose X-rays promoted both.

It is reported that preirradiation of X-rays decreases the incidence of brain tumors in rats exposed in utero [3]. This protective effect of preirradiation appears to correspond to the inductive effect of ionizing irradiation on O^6-alkylguanine alkyltransferase (ATase), which protects cells from G to A mutation. Induction of ATase by irradiation has been frequently observed in several tissues both in vitro and in vivo [4,5]. Our results, showing that 0.2 Gy X-rays combined with ENU decreased the G to A transition, suggest increased activity of ATase by radiation.

On the other hand, high-dose radiation can kill the target cells, thereby providing an environment for the surviving cells to expand. Irradiation of thymic epithelial cells enhances interleukin (IL)-7 production, and thymocytes at the preleukemic stage proliferate more vigorously in response to IL-7 [6,7]. We previously found frequent mutations of *Ikaros* in X-ray- and ENU-induced thymic lymphomas [8–10]. T cells with reduced or dominant-negative Ikaros activity, which may result from either a lack of or a point mutation in the zinc finger responsible for DNA binding, exhibit a greater proliferative response to IL-2 [11]. Taken together, these results suggest that high-dose radiation provides a thymic microenvironment ripe for the occurrence of prelymphoma cells, which harbor growth-advantageous mutations following ENU treatment.

In conclusion, low-dose (0.2 Gy) X-rays reduce not only the frequency of spontaneously occurring but also ENU-induced mutations, suggestive of an adaptive response. Low-dose X-rays also reduce the clonal expansion of cells following ENU treatment, whereas 1.0 Gy X-rays accelerate cell expansion. Thus, low- and high-dose radiation plays different roles in lymphomagenesis when combined with ENU exposure.

Conclusion

Combined exposure of carcinogens is a characteristic of ordinary human life. The dose of radiation is a critical factor to determine the mode of combined effect of radiation and ENU.

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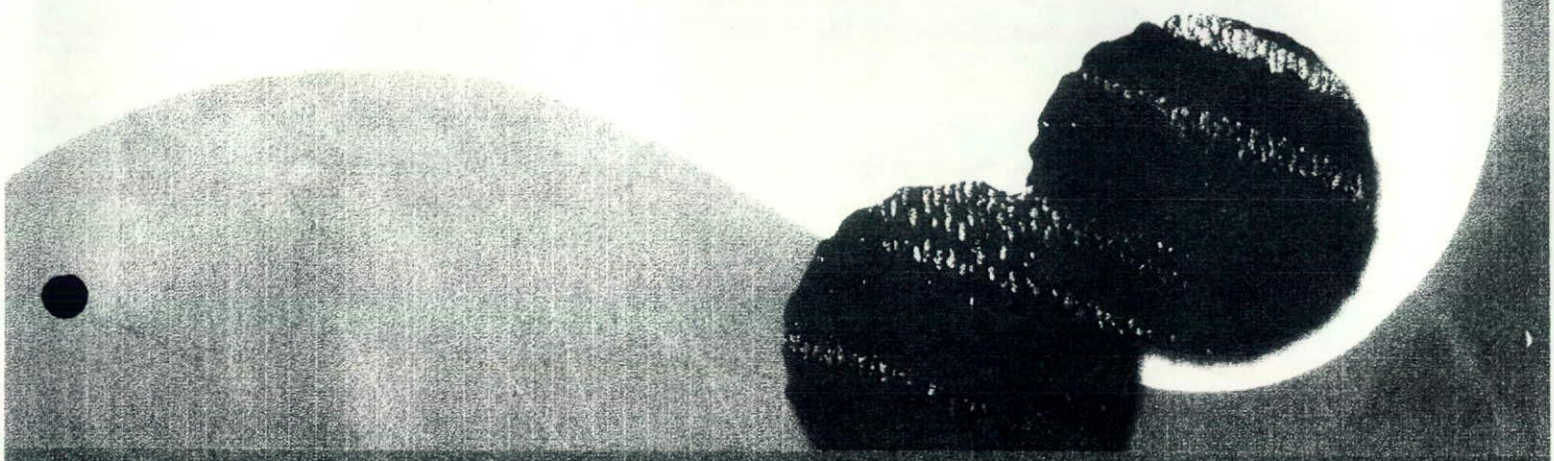

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