Table 73. イソフタル酸ジメチルのマトリックス案

| テスト項目 | 必須DATA | SIPM | SIPE | SIPA |
|---|---|---|---|---|
| 構造式 | | | | |
| CAS No. | | 3965-55-7 | 24019-46-3 （主成分） | 6362-79-4 |
| 分子量 | | 297 | 357 （主成分） | 269 |
| 物理化学性状 | | | | |
| 融点 | ○ | RA | ○ | RA |
| 沸点 | ○ | RA | ○ | RA |
| 密度 | | | | |
| 蒸気圧 | ○ | | | |
| 分配係数：n-ｵｸﾀﾉｰﾙ/水 | ○ | | | |
| 水溶解度 | ○ | ○ | ○ | ○ |
| 解離定数 | ○ | | | |
| 引火点 | | | | |
| 環境運命と経路 | | | | |
| 安定性　光分解 | ○ | ○ | RA | RA |
| 安定性　水中安定性 | ○ | | | |
| 分配経路を含む媒体間の移動と分配 | ○ | ○ | RA | RA |
| 環境ﾓﾆﾀﾘﾝｸﾞﾃﾞｰﾀ | | | | |
| 好気性生分解性 | ○ | ○ | ○ | RA |
| BOD/COD | | | | |
| 生物濃縮 | | | | |
| 生態毒性 | | | | |
| 急性毒性(魚類) | ○ | RA | ○ | ○ |
| 急性毒性(ﾐｼﾞﾝｺ) | ○ | RA | ○ | ○ |
| 藻類への急性毒性 | ○ | RA | ○ | ○ |
| ﾊﾞｸﾃﾘｱ等への急性毒性 | | | | |
| 水生生物への慢性毒性 | | | | |
| 陸生生物への毒性 | | | | |
| ヒト健康毒性 | | | | |
| 急性経口毒性 | ○ | 試験実施 | | |
| 急性吸入毒性 | ○ | | | |
| 急性経皮毒性 | ○ | | | |
| 反復投与毒性 | ○ | RA | ○ | ○ |
| 遺伝毒性in vitro遺伝子突然変異 | ○ | RA | ○ | ○ |
| 遺伝毒性in vitro染色体異 | ○ | RA | ○ | ○ |
| 遺伝毒性　その他 | | | | |
| 発癌性 | | | | |
| 受胎能 | ○ | 試験実施 | RA | RA |
| 発生毒性 | ○ | 試験実施 | RA | RA |
| 皮膚刺激性/腐食性 | | | | |
| 皮膚感作性 | | | | |
| 人暴露の情報 | | | | |

○：データ取得　RA:ﾘｰﾄﾞｱｸﾛｽ

Table 74. メトキシエチルアクリラートのマトリックス案

| テスト項目 | 必須 DATA | テトラメトキシシラン TMS | ケイ酸四ナトリウム塩 既存点検物質 | Silicic acid, sodium salt OECD/SIDS | Silicic acid, disodium salt OECD/SIDS | メタノール OECD/SIDS | トリメトキシシラン TriMS OECD/SIDS | テトラエトキシシラン TES OECD/SIDS |
|---|---|---|---|---|---|---|---|---|
| 構造式 | | | | | | $CH_3-OH$ | | |
| CAS No. | | 681-84-5 | 13472-30-5 | 1344-09-8 | 6834-92-0 | 67-56-1 | 2487-90-3 | 78-10-4 |
| 分子量 | | 152.25 | | | | | | |
| 物理化学性状 | | | | | | | | |
| 融点 | ○ | ○ | | ○ | ○ | ○ | ○ | ○ |
| 沸点 | ○ | ○ | | | | ○ | ○ | ○ |
| 密度 | | ○ | | ○ | ○ | ○ | | ○ |
| 蒸気圧 | ○ | ○ | | ○ | ○ | ○ | ○ | ○ |
| 分配係数: n-オクタノール/水 | ○ | ○ | | ○ | ○ | ○ | ○ | ○ |
| 水溶解度 | ○ | ○ | | ○ | ○ | ○ | ○ | ○ |
| 解離定数 | ○ | | | | | | | |
| 引火点 | | | | | | | | |
| 環境運命と経路 | | | | | | | | |
| 安定性 光分解 | ○ | ○ | | ○ | ○ | ○ | ○ | ○ |
| 安定性 水中安定性 | ○ | ○ | | ○ | ○ | ○ | | ○ |
| 分配経路を含む媒体間の移動と分配 | ○ | | | | | | | |
| 環境モニタリングデータ | | | | | | | | |
| 好気性生分解性 | ○ | ○ | | ○ | ○ | ○ | ○ | ○ |
| BOD/COD | | | | | | | | |
| 生物濃縮 | | | | | | | | |
| 生態毒性 | | | | | | | | |
| 急性毒性(魚類) | ○ | RA | 試験中 | ○ | ○ | ○ | ○ | ○ |
| 急性毒性(ミジンコ) | ○ | RA | 試験中 | ○ | | ○ | ○ | ○ |
| 藻類への急性毒性 | ○ | RA | 試験中 | ○ | | ○ | ○ | ○ |
| バクテリア等への急性毒性 | | | | | | | | |
| 水生生物への慢性毒性 | | | | | | | | |
| 陸生生物への毒性 | | | | | | | | |
| ヒト健康毒性 | | | | | | | | |
| 急性経口毒性 | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| 急性吸入毒性 | ○ | ○ | | | | ○ | ○ | ○ |
| 急性経皮毒性 | ○ | ○ | | | | ○ | ○ | ○ |
| 反復投与毒性 | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| 遺伝毒性in vitro遺伝子突然変異 | ○ | RA | ○ | ○ | ○ | | ○ | ○ |
| 遺伝毒性in vitro染色体異常 | ○ | RA | ○ | ○ | ○ | | ○ | ○ |
| 遺伝毒性 その他 | | | | | | | | |
| 発癌性 | | | | | | | | |
| 受胎能 | ○ | RA | | ○ | ○ | ○ | ○ | ○ |
| 発生毒性 | ○ | RA | ○ | | ○ | ○ | | ○ |
| 皮膚刺激性/腐食性 | | | | | | | | |
| 皮膚感作性 | | | | | | | | |
| 人暴露の情報 | | | | | | | | |

○:データ取得 RA:リードアクロス

Table 75. 脂肪酸クロライドのマトリックス案

| テスト項目 | 必須DATA | オクタン酸(C8脂肪酸) | デカン酸(C10脂肪酸) | ラウリン酸(C12脂肪酸) | ミリスチン酸(C14脂肪酸) | パルミチン酸(C16脂肪酸) | ステアリン酸(C18脂肪酸) | ドコサン酸(C22脂肪酸) | 塩化水素 |
|---|---|---|---|---|---|---|---|---|---|
| 構造式 | | $CH_3(CH_2)_5COOH$ | $CH_3(CH_2)_8COOH$ | $CH_3(CH_2)_{10}COOH$ | $CH_3(CH_2)_{12}COOH$ | $CH_3(CH_2)_{14}COOH$ | [illegible] | $CH_3(CH_2)_{20}COOH$ | HCl |
| CAS No. | | 124-07-2 | 334-48-5 | 143-07-7 | 544-63-8 | 57-10-3 | 57-11-4 | 112-85-6 | 7647-01-0 |
| 分子量 | | 144.00 | 172.26 | 200.00 | 228.00 | 256.00 | 284.50 | 340.00 | 36.50 |
| 物理化学性状 | | | | | | | | | |
| 融点 | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| 沸点 | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| 密度 | | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| 蒸気圧 | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| 分配係数：n-オクタノール/水 | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| 水溶解度 | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | |
| 解離定数 | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| 引火点 | | | | | | | | | |
| 環境運命と経路 | | | | | | | | | |
| 安定性　光分解 | ○ | | | | | | | ○ | ○ |
| 安定性　水中安定性 | ○ | | | | | | | ○ | ○ |
| 分配経路を含む媒体間の移動と分配 | ○ | | | ○ | ○ | ○ | | ○ | ○ |
| 環境モニタリングデータ | | | | | | | | | |
| 好気性生分解性 | ○ | | | | | ○ | ○ | ○ | |
| BOD/COD | | | | | | | | | |
| 生物濃縮 | | | | | | | | | |
| 生態毒性 | | | | | | | | | |
| 急性毒性(魚類) | ○ | | ○ | ○ | ○ | ○ | | ○ | ○ |
| 急性毒性(ミジンコ) | ○ | | ○ | ○ | ○ | ○ | | ○ | ○ |
| 藻類への急性毒性 | ○ | | ○ | ○ | ○ | ○ | | ○ | ○ |
| バクテリア等への急性毒性 | | | | | | | | | |
| 水生生物への慢性毒性 | | | | | | | | ○ | |
| 陸生生物への毒性 | | | | | | | | | |
| ヒト健康毒性 | | | | | | | | | |
| 急性経口毒性 | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| 急性吸入毒性 | ○ | | | | | | | | |
| 急性経皮毒性 | ○ | | | | | | | | |
| 反復投与毒性 | ○ | | | | | | ○ | ○ | ○ |
| 遺伝毒性in vitro遺伝子突然変異 | ○ | | | | | | | ○ | ○ |
| 遺伝毒性in vitro染色体異常 | ○ | ○ | ○ | | ○ | ○ | | ○ | ○ |
| 遺伝毒性　その他 | | | | | | | | | ○ |
| 発癌性 | | | | | | ○ | | | ○ |
| 受胎能 | ○ | 試験実施 | | | | | | ○ | |
| 発生毒性 | ○ | 試験実施 | | | | | | ○ | |
| 皮膚刺激性/腐食性 | | | | | | | | | ○ |
| 皮膚感作性 | | | | | | | | | ○ |
| 人暴露の情報 | | | | | | | | | |

○：データ取得

## Table 76. Pートルエンスルホン酸のマトリックス案

| テスト項目 | 必須 DATA | p-トルエンスルホン酸・水和物 | p-トルエンスルホン酸 | p-トルエンスルホン酸ナトリウム |
|---|---|---|---|---|
| | | | US チャレンジ | |
| 構造式 | | | | |
| CAS No. | | 6192-52-5 | 104-15-4 | 657-84-1 |
| 分子量 | | | | |
| **物理化学性状** | | | | |
| 融点 | ○ | ○ | ○ | ○ |
| 沸点 | ○ | ○ | ○ | ○ |
| 密度 | | | | |
| 蒸気圧 | ○ | ○ | ○ | ○ |
| 分配係数：n-オクタノール/水 | ○ | ○ | ○ | ○ |
| 水溶解度 | ○ | ○ | ○ | ○ |
| 解離定数 | ○ | ○ | ○ | ○ |
| 引火点 | | | | |
| **環境運命と経路** | | | | |
| 安定性　光分解 | ○ | ○ | ○ | ○ |
| 安定性　水中安定性 | ○ | | ○ | ○ |
| 分配経路を含む媒体間の移動と分配 | ○ | ○ | ○ | ○ |
| 環境モニタリングデーター | | | | |
| 好気性生分解性 | ○ | RA | ○ | ○ |
| BOD/COD | | | | |
| 生物濃縮 | | | | ○ |
| **生態毒性** | | | | |
| 急性毒性(魚類) | ○ | RA | ○ | ○ |
| 急性毒性(ミジンコ) | ○ | RA | ○ | ○ |
| 藻類への急性毒性 | ○ | RA | ○ | ○ |
| バクテリア等への急性毒性 | | | | |
| 水生生物への慢性毒性 | | | | ○ |
| 陸生生物への毒性 | | | | |
| **ヒト健康毒性** | | | | |
| 急性経口毒性 | ○ | RA | ○ | ○ |
| 急性吸入毒性 | ○ | | | |
| 急性経皮毒性 | ○ | | | |
| 反復投与毒性 | ○ | RA | ○ | ○ |
| 遺伝毒性in vitro遺伝子突然変異 | ○ | RA | ○ | ○ |
| 遺伝毒性in vitro染色体異常 | ○ | RA | ○ | ○ |
| 遺伝毒性　その他 | | | | |
| 発癌性 | | | | |
| 受胎能 | ○ | RA | | ○ |
| 発生毒性 | ○ | RA | | ○ |
| 皮膚刺激性/腐食性 | | | | |
| 皮膚感作性 | | | | |
| 人暴露の情報 | | | | |

○：データ取得　RA:リードアクロス

F.研究発表

1. 論文発表

Yatagai, F., Umebayashi, Y., Honma, M., Sugasawa, K., Takayama, Y., and Hanaoka, F. Mutagenic radioadaptation in a human lymphoblastoid cell line. Mutat. Res., 638, 48-55 (2008)

Yasui, M., Suenaga, E., Koyama, N., Masutani.C., Hanaoka, F., Gruz, P., Shibutani, S., Nohmi T., Hayashi, M., and Honma, M. Miscoding properties of 2'-deoxyinosine, a nitric oxide-derived DNA Adduct, during translesion synthesis catalyzed by human DNA polymerases. J Mol Biol, 377, 1015-1023 (2008)

Yatagai, F., Suzuki, M., Ishioka, N., Ohmori, H., Honma, M. Repair of I-SceI Induced DSB at a specific site of chromosome in human cells: influence of low-dose, low-dose-rate gamma-rays. Radiat Environ Biophys 47, 439-44 (2008)

Nhomi, T., Toyoda-Hokaiwado, N., Yamada, M., Masumura, K., Honma, M., and Fukushima, S. International Symposium on Genotoxic and Carcinogenic Thresholds. Genes and Environment, 30, 101-107 (2008)

本間正充；遺伝毒性物質に閾値はあるのか？ファルマシア 45, 143-148 (2009)

Ema, M., Fujii, S., Hirata-Koizumi, M., and Matsumoto, M. (2008). Two-generation reproductive toxicity study of the flame retardant hexabromocyclododecane in rats. *Reprod Toxicol* **25**, 335-351.

Ema, M., Fukunishi, K., Hirose, A., Hirata-Koizumi, M., Matsumoto, M., and Kamata, E. (2008). Repeated-dose and reproductive toxicity of the ultraviolet absorber 2-(3',5'-di-tert-butyl-2'-hydroxyphenyl)-5-chlorobenzotriazole in rats. *Drug Chem Toxicol* **31**, 399-412.

Ema, M., Fukunishi, K., jii, S., Matsumoto, M., Hirata-Koizumi, M., and Ihara, T. Developmental toxicity of dibutyltin dichloride given on three consecutive days during organogenesis in cynomolgus monkeys. *Drug Chem Toxicol* in press.

Harada, T., Kimura, E., Hirata-Koizumi, M., Hirose, A., Kamata, E., and Ema, M. (2008). Reproductive and developmental toxicity screening study of 4-aminophenol in rats. *Drug Chem Toxicol* **31**, 473-486.

Hirata-Koizumi, M., Matsuyama, T., Imai, T., Hirose, A., Kamata, E., and Ema, M. (2008). Gender-related difference in the toxicity of ultraviolet absorber 2-(3',5'-di-tert-butyl-2'-hydroxyphenyl)-5-chlorobenzotriazole in rats. *Drug Chem Toxicol* **31**, 383-398.

Hirata-Koizumi, M., Matsuyama, T., Imai, T., Hirose, A., Kamata, E., and Ema, M. (2008). Lack of gender-related difference in the toxicity of 2-(2'-hydroxy-3',5'-di-tert-butylphenyl)benzotriazole in preweaning rats. *Drug Chem Toxicol* **31**, 275-287.

Hirata-Koizumi, M., Matsuno, K., Kawabata, M., Yajima, K., Matsuyama, T., Hirose, A., Kamata, E., and Ema, M. Gender-related difference in the toxicity of 2-(2'-hydroxy-3',5'-di-*tert*-butylphenyl)benzotriazole in rats: relationship to the plasma Concentration, in vitro hepatic metabolism and effects on hepatic metabolizing enzyme activity. *Drug Chem Toxicol* in press.

Matsumoto, M., Hirose, A., and Ema, M. (2008) Review of testicular toxicity of dinitrophenolic compounds, 2-sec-butyl-4,6-dinitrophenol, 4,6-dinitro-o-cresol and 2,4-dinitrophenol. *Reprod Toxicol* **26**, 185-190.

Takahashi, M., Sunaga, M., Hirata-Koizumi, M., Hirose, A., Kamata, E., and Ema, M. Reproductive and developmental toxicity screening study of 2,4-dinitrophenol in rats. *Environ Toxicol* in press.

高橋美加、松本真理子、宮地繁樹、菅野誠一郎、菅谷芳雄、広瀬明彦、鎌田栄一、江馬 眞 (2008) OECD化学物質対策の動向（第14報）－第23回、第24回OECD高生産量化学物質初期評価会議（2006年済州、2007年パリ）、化学生物総合管理, 4, 225-236.

松本真理子、宮地繁樹、菅谷芳雄、江馬 眞、広瀬明彦 (2009) OECD高生産量化学物質点検プログラム：第26回初期評価会議概要、化学生物総合管理, 4, 237-245.

Kobayashi, K., KS Pillai, Y. Sakuratani, T. Abe, E. Kamata and M.Hayashi (2008) Evaluation of statistical tools used in short-term repeated dose administration toxicity studies with rodent, J. Toxicol. Sci., 33, 97-104.

Lorge, E., M. Hayashi, S. Albertini, and D. Kirkland (2008) Comparison of different methods for an accurate assessment of cytotoxicity in the in vitro micronucleus test I. Theoretical aspects, Mutat. Res., 655, 1-3.

2. 学会発表

本間正充: 医薬品に不純物として含まれる遺伝毒性物質の分類と許容量　第35回日本トキシコロジー学会学術年会(2008.6)

本間正充: ICH における新しい遺伝毒性試験ガイドライン (S2R1) と試験実施タイミング　第35回日本トキシコロジー学会学術年会(2008.6)

Honma, M. Ultimate Threshold and Genetic Consequence of A Single Double Strand Break in Human Cells. International Symposium on Genotoxic and Carcinogenic Thresholds (2008.7)

Honma, M. Genome Mapping of Damaged Chromosome Regions Induced by Ionizing Irradiation Using DNA Microarray Analysis. 38th European Environmental Mutagen Society (2008.9)

鈴木孝昌、小木美恵子、小原有弘、本間正充、田邊思帆里、山口照英：SNP およびCGH マイクロアレイを用いた c-myc 遺伝子増幅に関する詳細解析　第67回日本癌学会総会(2008.10)

谷田貝文夫、菅澤薫、榎本秀一、本間正充：DSB 修復効率からの適応応答の追求　日本放射線影響学会第51回大会(2008.11)

本間正充：DNA 二本鎖切断修復と遺伝的不安定性　日本環境変異原学会第37回大会(2008.12)

安井　学、本間正充：ヒトリンパ球細胞のゲノム内に導入させた 8-オキソグアニン1分子の突然変異誘発能　日本環境変異原学会第37回大会(2008.12)

小山直己、木村葵、安井学、高見成昭、高橋美和、今井俊夫、山本歩、汲田和歌子、増村健一、増田修一、木苗直秀、松田知成、能美健彦、本間正充：ライフステージを考慮したアクリルアミドの多臓器遺伝毒性評価　日本環境変異原学会第37回大会(2008.12)

斉藤美香、松藤寛、千野誠、林真、本間正充、山形一雄：過酸化水素によって誘導されたヒトリンパ芽球細胞TK6の細胞増殖と遺伝毒性に対する天然抗酸化物質の保護効果　日本環境変異原学会第37回大会(2008.12)

木村葵、坂本浩子、西郷和彦、洲加本孝幸、本間正充：*In vitro* コメットアッセイプロトコールの検証　日本環境変異原学会第37回大会(2008.12)

Wang, J., Sawyer, J., Honma, M., Chen, T., and Moore, M. The Mouse Lymphoma Assay detects recombination, deletion, and aneuploidy.　日本環境変異原学会第37回大会(2008.12)

谷田貝文夫、高橋昭久、本間正充、鈴木ひろみ、大森克徳、関真也、橋爪藤子、島津徹、榎本秀一、大西武雄、石岡憲昭：宇宙実験：放射線影響の LOH 検出系による解析　日本環境変異原学会第37回大会(2008.12)

鈴木孝昌、小泉朋子、本間正充、中嶋圓、濱田修一、渡辺貴志、降旗千恵：トキシコゲノミクスに関する JEMS/MMS 共同研究II：遺伝子傷害性発癌物質の迅速スクリーニング系としての TaqMan Low Density Array の評価　日本環境変異原学会第37回大会(2008.12)

本間正充、櫻庭真弓、汲田和歌子、林　真：DNA マイクロアレイによる放射線損傷領域のゲノムマッピング　日本環境変異原学会第37回大会(2008.12)

中嶋圓、鈴木雅也、田中仁、本間正充、林真：*In vitro* コメットアッセイ国際バリデーションデータ解析に関する一考察　日本環境変異原学会第37回大会(2008.12)

安井学、Suzuki, N.、本間正充、Shibutani, S.：一酸化窒素によって形成する DNA 付加体の誤塩基対形成メカニズム　第31回分子生物学会(2008. 12)

Hasegawa, R., Hirata-Koizumi, M., and Hirose, A. Proposal of new uncertainty factor application to derive tolerable daily intake. The 45th Congress of the European Societies of Toxicology (October 2008, Rhodes, Greece).

Hirose, A., Ishiwa, S., Ciloy, J. M., Takahashi, M., Hirata-Koizumi, M., Kamata, E., Ono, A., Ema, M., and Hayashi, M. Development of in silico hepatotoxicity predicting system on sub-acute repeated dose toxicity test for industrial chemicals. The 45th Congress of the European Societies of Toxicology (October 2008, Rhodes, Greece).

Hirose, A., Schlueter, T., Matsumoto, M., Hirata-Koizumi, M., Kamata, E., Kremoser, C., and Ema, M. Modulation of Nuclear Receptor Cofactor Recruitment by Tributyltin and Dibutyltin in Gal4 Assays. Dioxin2008 (August 2008, Birmingham, UK).

Hirata-Koizumi, M., Noda, A., Hirose, A., Kamata, E., and Ema, M. Screening study for reproductive and developmental toxicity of tetrahydrofurfuryl alcohol in rats. The 45th Congress of the European Societies of Toxicology (October 2008, Rhodes, Greece).

Nishimura, T., Shimizu, K., Kubota, R., Tahara, M.,

effects of fullerene (C60) exposed using liposome in HepG2 cells. The 45th Congress of the European Societies of Toxicology (October 2008, Rhodes, Greece).

平田睦子, 離乳前ラットにおける紫外線吸収剤 2-(2'-hydroxy-3',5'-di-*tert*-butylphenyl) benzotriazolの毒性影響. 第48回日本先天異常学会学術集会 (2008年6月, 東京).

平田睦子, 松野喜代美, 川端光彦, 矢島加奈子, 松山隆史, 広瀬明彦, 鎌田栄一, 江馬眞, 2-(2'-Hydroxy-3',5'-di-tert-butylphenyl)benzotriazole (HDBB)の毒性 —血中濃度及び肝薬物代謝酵素活性に対する影響. 第35回日本トキシコロジー学会学術年会 (2008年6月, 東京).

平田睦子, 野田篤, 広瀬明彦, 鎌田栄一, 江馬眞, Tetrahydrofurfuryl alcoholの簡易生殖毒性試験. 第48回日本先天異常学会学術集会 (2008年6月, 東京).

緒方英博, 平田睦子, 今井俊夫, 広瀬明彦, 鎌田栄一, 江馬眞, 2-(2'-Hydroxy-3',5'-di-tert-butylphenyl)benzotriazole (HDBB)の52週間反復投与毒性試験. 第35回日本トキシコロジー学会学術年会 (2008年6月, 東京).

T Ueda, T Inaba, Mkanbe, H Mashiko, C Wakui, M Hayashi, Increase of Structural-chromosomal Aberrations and Micronuclei Caused by Mitomycin C Treatment in Embryo Cells of Zebrafish. 第37回日本環境変異原学会(2008年12月, 沖縄)

Jin Tanaka, Daisuke Mukai, Kanako Iwakura, Takefumi Kimoto, Daishiro Miura, Madoka Nakajima, Makoto Hayashi, Validation of a Gene Mutation Assay using a Pig-A gene. 第37回日本環境変異原学会(2008年12月, 沖縄)

Makoto Hayashi, Regulatory Issues on Pharmaceutical Chemicals—Revision of ICH S2—.第37回日本環境変異原学会(2008年12月, 沖縄)

林真 化学物質の安全性評価–*in silico*への挑戦. 薬物動態談話会特別例会(2008年11月, 浜松)

Makoto Hayashi, Threshold of Genotoxicity . International Symposium on Genotoxic and Carcinogenic Thresholds (2008年7月, 東京)

G. 知的財産権の出願・登録状況 (予定を含む)

1. 特許取得
   (該当なし)
2. 実用新案登録
   (該当なし)
3. その他
   (該当なし)

H. 健康危機情報

特になし

別添４

研究成果の刊行に関する一覧表レイアウト

**書籍**

| 著者名 | 論文ﾀｲﾄﾙ名 | 書籍全体の編集者名 | 書籍名 | 出版社名 | 出版値 | 出版年 | ﾍﾟｰｼﾞ |
|---|---|---|---|---|---|---|---|
| | | | | | | | |
| | | | | | | | |

**雑誌**

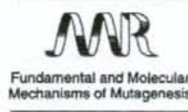
| 発表者名 | 論文ﾀｲﾄﾙ名 | 発表誌名． | 巻号 | ﾍﾟｰｼﾞ | 出版年 |
|---|---|---|---|---|---|
| Yatagai, F., Umebayashi, Y., Honma, M., Sugasawa, K., Takayama, Y., and Hanaoka, F. | Mutagenic radioadaptation in a human lymphoblastoid cell line. | Mutat. Res., | 638 | 48-55 | 2008 |
| Yasui, M., Suenaga, E., Koyama, N., Masutani.C., Hanaoka, F., Gruz, P., Shibutani, S., Nohmi T., Hayashi, M., and Honma, M. | Miscoding properties of 2'-deoxyinosine, a nitric oxide-derived DNA Adduct, during translesion synthesis catalyzed by human DNA polymerases. | J Mol Biol, | 377 | 1015-1023 | 2008 |
| Yatagai, F., Suzuki, M., Ishioka, N., Ohmori, H., Honma, M. | Repair of I-SceI Induced DSB at a specific site of chromosome in human cells: influence of low-dose, low-dose-rate gamma-rays. | Radiat Environ Biophys | 47 | 439-444 | 2008 |
| Nhomi, T., Toyoda-Hokaiwado, N., Yamada, M., Masumura, K., Honma, M., and Fukushima, S. | International Symposium on Genotoxic and Carcinogenic Thresholds. | Genes and Environment, | 30 | 101-107 | 2008 |
| 本間正充 | 遺伝毒性物質に閾値はあるのか？ | ﾌｧﾙﾏｼｱ | 45 | 143-148 | 2008 |
| Ema, M., Fujii, S., Hirata-Koizumi, M., and Matsumoto, M. | Two-generation reproductive toxicity study of the flame retardant hexabromocyclododecane in rats. | *Reprod Toxicol* | 23 | 335-351 | 2008 |
| Ema, M., Fukunishi, K., Hirose, A., Hirata-Koizumi, M., Matsumoto, M., and Kamata, E. | Repeated-dose and reproductive toxicity of the ultraviolet absorber 2-(3',5'-di-*tert*-butyl-2'-hydroxyphenyl)-5-chlorobenzo triazole in rats. | *Drug Chem Toxicol* | 31 | 399-412 | 2008 |
| Ema, M., Fukunishi, K., jii, S., Matsumoto, M., Hirata-Koizumi, M., and Ihara, T. | Developmental toxicity of dibutyltin dichloride given on three consecutive days during organogenesis in cynomolgus monkeys. | *Drug Chem Toxicol* | in press | | |
| Harada, T., Kimura, E., Hirata-Koizumi, M., Hirose, A., Kamata, E., and Ema, M. | Reproductive and developmental toxicity screening study of 4-aminophenol in rats. | *Drug Chem Toxicol* | 31 | 473-486 | 2008 |
| Hirata-Koizumi, M., Matsuyama, T., Imai, T., Hirose, A., Kamata, | Gender-related difference in the toxicity of ultraviolet absorber 2-(3',5'-di-*tert*-butyl-2'-hydroxyphenyl)-5-chlorobenzo | *Drug Chem Toxicol* | 31 | 383-398 | 2008 |

| | | | | | |
|---|---|---|---|---|---|
| E., and Ema, M. | triazole in rats. | | | | |
| Hirata-Koizumi, M., Matsuyama, T., Imai, T., Hirose, A., Kamata, E., and Ema, M. | Lack of gender-related difference in the toxicity of 2-(2'-hydroxy-3',5'-di-*tert*-butylphenyl)benzotriazole in preweaning rats. | *Drug Chem Toxicol* | 31 | 275-287 | 2008 |
| Hirata-Koizumi, M., Matsuno, K., Kawabata, M., Yajima, K., Matsuyama, T., Hirose, A., Kamata, E., and Ema, M. | Gender-related difference in the toxicity of 2-(2'-hydroxy-3',5'-di-*tert*-butylphenyl)benzotriazole in rats: relationship to the plasma Concentration, in vitro hepatic metabolism and effects on hepatic metabolizing enzyme activity. | *Drug Chem Toxicol* | in press | | |
| Matsumoto, M., Hirose, A., and Ema, M. | Review of testicular toxicity of dinitrophenolic compounds, 2-sec-butyl-4,6-dinitrophenol, 4,6-dinitro-o-cresol and 2,4-dinitrophenol. | *Reprod Toxicol* | 26 | 185-190 | 2008 |
| Takahashi, M., Sunaga, M., Hirata-Koizumi, M., Hirose, A., Kamata, E., and Ema, M. | Reproductive and developmental toxicity screening study of 2,4-dinitrophenol in rats. | *Environ Toxicol* | in press | | |
| 高橋美加、松本真理子、宮地繁樹、菅野誠一郎、菅谷芳雄、広瀬明彦、鎌田栄一、江馬　眞 | OECD化学物質対策の動向（第14報）－第23回、第24回OECD高生産量化学物質初期評価会議（2006年済州、2007年パリ） | 化学生物総合管理 | 4 | 225-236 | 2008 |
| 松本真理子、宮地繁樹、菅谷芳雄、江馬眞、広瀬明彦 | OECD高生産量化学物質点検プログラム：第26回初期評価会議概要 | 化学生物総合管理 | 4 | 237-245 | 2008 |
| Kobayashi, K., KS Pillai, Y. Sakuratani, T. Abe, E. Kamata and M.Hayashi | Evaluation of statistical tools used in short-term repeated dose administration toxicity studies with rodent, | J. Toxicol. Sci., | 33 | 97-104 | 2008 |
| Lorge, E., M. Hayashi, S. Albertini, and D. Kirkland | Comparison of different methods for an accurate assessment of cytotoxicity in the in vitro micronucleus test I. Theoretical aspects | Mutat. Res., | 655 | 1-3 | 2008 |

# Ⅲ．研究成果の刊行物・別刷

Available online at www.sciencedirect.com

Mutation Research 638 (2008) 48–55


Fundamental and Molecular Mechanisms of Mutagenesis
www.elsevier.com/locate/molmut
Community address: www.elsevier.com/locate/mutres


# Mutagenic radioadaptation in a human lymphoblastoid cell line


Fumio Yatagai [a,*], Yukihiro Umebayashi [a], Masamitsu Honma [b], Kaoru Sugasawa [c], Yuko Takayama [a], Fumio Hanaoka [d]

[a] *Advanced Development and Support Center, The Institute of Physical and Chemical Research (RIKEN), Saitama 351-0198, Japan*
[b] *Division of Genetics and Mutagenesis, National Institute of Health Sciences, Tokyo 158-8501, Japan*
[c] *Genome Damage Response Research Unit, The Institute of Physical and Chemical Research (RIKEN), Saitama 351-0198, Japan*
[d] *Graduate Program, Frontiers in Biosciences, Osaka University, Osaka 565-0871, Japan*



Received 6 April 2007; received in revised form 15 August 2007; accepted 22 August 2007
Available online 1 September 2007



**Abstract**

We investigated the mutagenic radioadaptive response of human lymphoblastoid TK6 cells by pretreating them with a low dose (5 cGy) of X-rays followed by a high (2 Gy) dose 6 h later. Pretreatment reduced the 2-Gy-induced mutation frequency (MF) of the *thymidine kinase* (*TK*) gene ($18.3 \times 10^{-6}$) to 62% of the original level ($11.4 \times 10^{-6}$). A loss of heterozygosity (LOH) detection analysis applied to the isolated $TK^-$ mutants revealed the mutational events as non-LOH (resulting mostly from a point mutation in the *TK* gene), hemizygous LOH (resulting from a chromosomal deletion), or homozygous LOH (resulting from homologous recombination (HR) between chromosomes). For non-LOH events, pretreatment decreased the frequency to 27% of the original level (from $7.1 \times 10^{-6}$ to $1.9 \times 10^{-6}$). cDNAs prepared from the non-LOH mutants revealed that the decrease was due mainly to the repression of base substitutions. The frequency of hemizygous LOH events, however, was not significantly altered by pretreatment. Mapping analysis of chromosome 17 demonstrated that the distribution and the extent of hemizygous LOH events were also not significantly influenced by pretreatment. For homozygous LOH events, pretreatment reduced the frequency to 61% of the original level (from $5.1 \times 10^{-6}$ to $3.1 \times 10^{-6}$), reflecting an enhancement in HR repair of DNA double-strand breaks. Our findings suggest that the radioadaptive response in TK6 cells follows mainly from mutations at the base-sequence level, not the chromosome level.


© 2007 Elsevier B.V. All rights reserved.



*Keywords:* Adaptive response; TK6 cells; LOH detection system


## 1. Introduction

An adaptive cellular response occurs when a mild stress applied before a challenging treatment with a DNA-damaging agent decreases the detrimental effects of the challenge. In radioadaptation, as it is usually defined, exposure to a low dose of ionizing radiation (IR) provides some protection against a high dose. Radioadaptation was first reported by Olivieri et al. [1], who showed that radiation delivered by labeling human lymphocytes with tritiated thymidine causes a decrease in the frequency of chromosomal aberrations induced by subsequent exposure to 15 Gy of IR. That discovery stimulated a series of studies in human lymphocytes and various mammalian cell lines (for review, see refs. [2,3]) and suggested that the adaptive response is an important defense mechanism, especially against low doses of IR. The molecular mechanisms involved, however, remain largely unknown [4–8], and cellular


* Corresponding author. Tel.: +81 48 467 9710; fax: +81 48 462 1426.
*E-mail address:* yatagai@postman.riken.go.jp (F. Yatagai).



0027-5107/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.mrfmmm.2007.08.014

responses such as the bystander effect, genetic instability and hyper-radiosensitivity seem tightly related to the adaptive response in a specific low-dose region. One of the hot subjects in recent adaptive response studies is the expression of the genes involved in the mechanism [8–10]. Another is the relationship between the adaptive response and the bystander effect [11–15]. In mammalian cells, for example, bystander mutagenesis may be suppressed by an adaptive response [11].

Following the report by Olivieri et al., reduced induction of both micronuclei and sister chromatid exchanges was shown in Chinese hamster V79 cells pre-exposed to low doses of γ-rays or $^3$H β-rays [16]. Subsequent studies reported similar radioadaptive responses, such as reduced mutation frequencies in human lymphocytes [17], mouse SR-1 cells [18] and human–hamster hybrid $A_L$ cells [19], an altered mutation spectrum in human–hamster hybrid $A_L$ cells [19], reduced micronucleus frequencies in human lymphocytes [5] and mouse embryo cells [20], and reduced deletions and rearrangements in human lymphoblast cells [21]. The mechanism underlying those radioadaptations may have been the induction of an efficient chromosome repair system by the priming radiation dose, and in fact, the efficiency of DNA double-strand break (DSB) repair in Chinese hamster V79 cells exposed to γ-rays is enhanced by a priming exposure of 5 cGy of γ-rays [22]. Furthermore, DSBs with either blunt or staggered ends, created by restriction enzymes, induce the adaptive response [3].

The human lymphoblastoid TK6 cell line, isolated by Skopek et al. [23], is heterozygous at the *thymidine kinase* (*TK*) locus. Honma's laboratory developed a loss of heterozygosity (LOH) detection system that can be used for molecular analysis of *TK* mutations as well as for detecting alterations at the chromosome level [24,25]. Using that methodology, we were able to detect IR effects at doses as low as 10 cGy [26–28]. Irradiation of TK6 cells with 10 cGy of X-rays clearly demonstrated radiation-specific types of LOH events or interstitial deletions in chromosome 17 [26]. We also observed more efficient induction of such events after 10 cGy irradiation with an accelerated carbon-ion (135 MeV/u) beam [27], and this was apparent in frozen cells exposed to the same carbon-ion beam [28]. These results strongly suggest that the interstitial deletions were the result of end-joining repair of IR-induced DSBs.

Because the radiation-sensitive LOH analysis system in TK6 cells is effective for detecting the fate of radiation-induced DNA double-strand breaks (DSBs), we use it here to see if the adaptive response could produce measurable changes in IR-induced genetic alterations. The results we obtained were not completely expected, but are interesting.

## 2. Materials and methods

### 2.1. Cell culture and adaptive treatment

The methodologies for the detection of *TK*-deficient mutants and the materials and methods used for cell culture and growth have been previously reported [26]. Briefly, TK6 cells were incubated in RPMI1640 medium supplemented with HAT to eliminate pre-existing $TK^-$ deficient mutants. The cells were then resuspended in fresh normal medium, and 6 ml cell suspension was dispensed into 6-cm diameter Petri dishes. The cells were pretreated ("primed") with 2.5, 5 or 10 cGy of X-rays (250 kVp) at a rate of 10 cGy/min, and placed in a 5% $CO_2$ humidified incubator. The cell concentration was adjusted to $8 \times 10^5$ cells/ml at the end of the post-irradiation incubation period of 1.5, 3, 6, 9 or 12 h. The cells were then challenged with 2 Gy X-rays (250 kVp) at 1 Gy/min. Non-primed irradiated cells treated in the same manner as the primed cells served as controls.

### 2.2. Survival assay and TK mutation assay

To determine the surviving fraction of the challenged cells, we measured the plating efficiency (PE) immediately after irradiation using the limiting dilution method. For mutation expression, we incubated the cells with non-selecting RPMI1640 medium for about 60 h following the X-ray challenge. We measured the PE of incubated cells similarly, determining the *TK* mutation frequency. To select $TK^-$ mutant clones, we seeded incubated cells into 96-well plates at $4 \times 10^4$ cells per well in RPMI1640 medium containing 4 μg/ml trifluorothymidine (TFT); we harvested the normally growing clones after 2 weeks and the slow growing clones after 4 weeks.

### 2.3. Determination of optimum irradiation conditions for mutagenic adaptation

To determine the optimum conditions for evoking the mutagenic radioadaptive-response, we tested the MF induced by 2 Gy at 0, 1.5, 3, 6, 9 and 12 h after a priming dose of 10 cGy, selected the optimum interval time, and then tested the MF induced by 2 Gy at that interval time after priming doses of 0, 2.5, 5 and 10 cGy.

### 2.4. LOH analysis of $TK^-$ mutants

Fig. 1 illustrates how we classified $TK^-$ mutants. We first determined *TK* LOH by PCR analysis of exons 4 and 7 [29]. If the PCR products of both were similar to those of the parental *TK* heterozygous cells, we classified the mutant

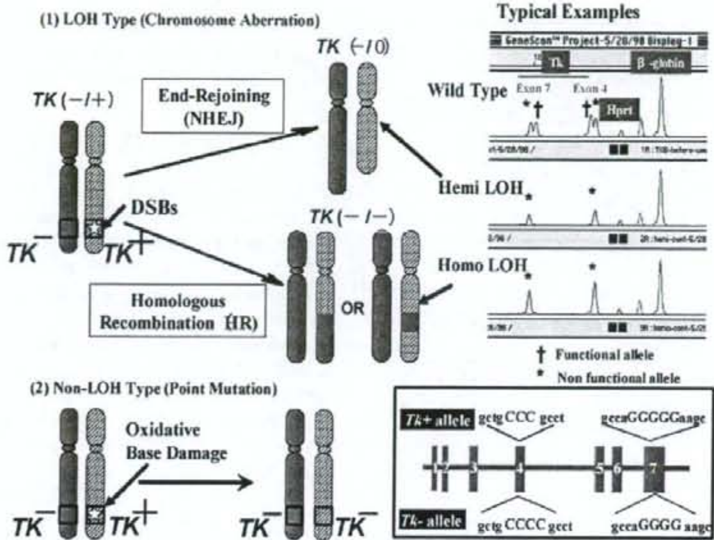



Fig. 1. LOH classifications of $TK^-$ mutants. The first step in the genetic analysis of selected $TK^-$ mutants was to judge whether there was a loss of *TK* heterozygosity (LOH). This was accomplished by PCR amplification of exons 4 and 7 regions of the *TK* locus. This step also distinguished between hemizygous LOH (loss of the functional *TK* allele) and homozygous LOH (replacement of the functional *TK* allele by a mutated $TK^-$ allele (see ref. [29]).

as a "non-LOH" mutant. We used the same technique to distinguish between hemizygous LOH (in which the functional *TK* allele is lost) and homozygous LOH (in which the functional *TK* allele is replaced by $TK^-$). To determine the extent and size of the deleted or substituted portions of the chromosome involved, we analyzed 11 microsatellite regions (D17S588, D17S1784, D17S785, D17S789, D17S802, D17S807, D17S928, D17S932, D17S1299, D17S1566 and THRA) on chromosome 17 using multiple PCR reactions as described previously [29]. The fine structure of the recovered $TK^-$ LOH mutations was determined by chromosome mapping analysis.

### 2.5. Base sequencing of non-LOH mutants

For a precise analysis of non-LOH mutants, we extracted RNA using Isogen (Nippon Gene, Japan), and obtained cDNA using a First-Strand cDNA Synthesis Kit, Amersham, USA). Following PCR amplification, the purified 807-bp fragments were sequenced by Takara Bio (Japan). The primers 5′-AGAGTACTCGGGTTCGTGAA-3′ and 5′-GCAGCATGCAGGGCAGCGTG-3′ (forward and reverse, respectively) were used for cDNA synthesis, PCR amplification and base sequencing [30]. To prevent the overestimation of mutational events, we counted identical mutations originating from a single irradiated dish as a single event.

Table 1a
*TK* mutation frequency (MF) at various time intervals between priming and challenging X-ray exposures (priming dose, 10 cGy; challenging dose, 2 Gy)

| Time interval (h) | 0 | 1.5 | 3 | 6 | 9 | 12 |
|---|---|---|---|---|---|---|
| *TK* MF ($\times 10^{-6}$) | 19.8 | 18.1 | 14.4 | 13.5 | 17.8 | 19.7 |

## 3. Results

### 3.1. Optimum conditions for mutagenic adaptation

For inducing an adaptive response to X-ray irradiation, the optimum interval between a 10-cGy priming dose and a 2-Gy challenging dose was 6 h (Table 1a), and the optimum priming dose 6 h prior to a 2-Gy challenging dose was 5 cGy (Table 1b). We therefore decided to characterize the induced *TK* mutants by repeating

Table 1b
*TK* mutation frequency at various priming X-ray doses (challenging dose, 2 Gy; interval between 2 exposures, 6 h)

| Priming X-ray dose (cGy) | 0 | 2.5 | 5 | 10 |
|---|---|---|---|---|
| *TK* MF ($\times 10^{-6}$) | 13.3 | 15.8 | 4.5 | 6.3 |

Table 2
Surviving fractions of primed and non-primed TK6 cells following challenge exposure to 2 Gy X-rays

| Experiment | Surviving fraction | |
|---|---|---|
| | Non-primed cells | Primed cells (5 cGy) |
| I | 0.043 | 0.047 |
| II | 0.047 | 0.070 |
| III | 0.049 | 0.040 |
| Mean ± S.D. | 0.046 ± 0.0031* | 0.052 ± 0.016* |

* $P = 0.58$; t-test.

Table 3
*TK* mutation frequency in primed and non-primed TK6 cells following challenge exposure to 2 Gy X-rays

| Experiment | *TK* mutation frequencies ($\times 10^{-6}$) | |
|---|---|---|
| | Non-primed cells | Primed cells (5 cGy) |
| I | 13.3 | 4.5 |
| II | 13.3 | 10.5 |
| III-a | 20.4 | 15.1 |
| III-b | 21.0 | 15.6 |
| Mean ± S.D. | 18.3 ± 4.3* | 11.4 ± 5.1* |

Experiments III-a and III-b were carried out concurrently with survival assay III, but they were independent mutation assays.
* $P = 0.020$; t-test.

our mutation experiments under those conditions (5 cGy followed 6 h later with 2 Gy).

### 3.2. Survival assay and TK mutation assay

Table 2 shows the surviving fraction, expressed as PE (2 Gy X-ray irradiated cells)/PE (unirradiated cells) of primed and unprimed cells immediately after the 2-Gy challenge exposure. Irradiation with the priming dose of 5 cGy did not influence the PE of unchallenged cells (data not shown). The effect of priming on survival after 2 Gy X-ray irradiation was 1.1 (0.052/0.046; $P = 0.58$, t-test). Thus, priming did not significantly affect survival after the challenge exposure.

On the other hand, priming did affect the *TK* MF induced by the challenge. Data from 4 independent experiments showed that priming reduced the MF to 62% of the unprimed MF ($P = 0.020$, t-test) (Table 3).

### 3.3. LOH analysis of $TK^-$ mutants

Table 4 shows the distributions of LOH classes among the isolated $TK^-$ mutants as determined by PCR analysis. We isolated non- and "small" LOH mutants (see Sections 3.4 & 3.5) as normal growth mutants in the first selection, except for a few cases. We isolated the remaining LOH mutants as slow growth mutants in the second selection. We estimated the pre-exposure effect from the proportion of each mutational event as follows: (i) $7.1 \times 10^{-6}$ to $1.9 \times 10^{-6}$ reduction in corresponding MF of non-LOH events, (ii) $6.4 \times 10^{-6}$ to $6.1 \times 10^{-6}$ change in corresponding MF of hemizygous LOH events and (iii) $5.1 \times 10^{-6}$ to $3.1 \times 10^{-6}$ reduction in corresponding MF of homozygous LOH events. Thus, the MF of a non-LOH event in primed cells was reduced to 27% of the non-primed MF. The induction of hemizygous events, on the other hand, was barely influenced by priming. As far as homozygous events go, their corresponding MF was reduced to 61% of the original level, which was similar to level of reduction in total MF (62%).

### 3.4. Analysis of LOH tracts on chromosome 17

Fig. 2 shows the deleted or replaced regions of chromosome 17 in each LOH mutant. Mutants reflected both type 1 and type 2 LOH events. Type 1 defines a terminal event; that is, the deleted or exchanged chromosome segment extends to the telomere marker (D17S928). Type 2 defines an interstitial deletion; the altered segment does not reach the telomere marker.

In the present study, most hemizygous LOH mutations, which are considered to be the result of DSB non-homologous end-joining (NHEJ) repair, reflected

Table 4
Distribution of mutational classes among the isolated *TK* mutants

| Mutational class | Number of identified mutants (Exp. I, II, III-a, III-b) [MF $\times 10^{-6}$] | |
|---|---|---|
| | Non-primed cells | Primed cells (5 cGy) |
| Non-LOH | 18 (5, 4, 6, 3) [7.1] | 8 (1, 3, 2, 2) [1.9] |
| LOH | | |
| Hemizygous | 15 (3, 3, 7, 2) [6.0] | 27 (8, 7[a], 5, 7) [6.4] |
| Homozygous | 13 (3, 4, 3, 3) [5.1] | 13 (2, 3, 5, 3) [3.1] |
| Total | 46 (11, 11, 16, 8) [18.3] | 48 (11, 13, 12, 12) [11.4] |

[a] One of the seven mutants was a mixed hemizygous/homozygous type.

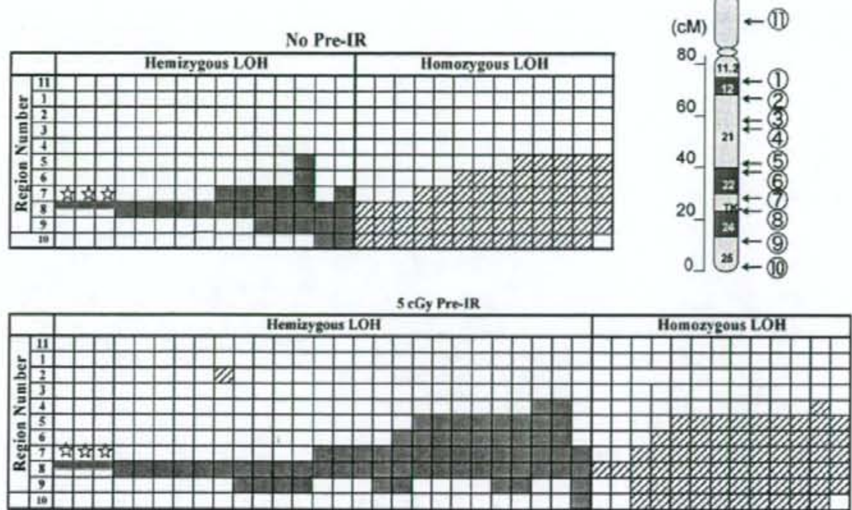



Fig. 2. Chromosome mapping of the LOH mutants. We analyzed the LOH mutants selected after a 2 Gy of challenging X-ray irradiation to determine the extent of the deleted or exchanged portions of the chromosome. The upper panel shows the profiles of 28 LOH mutants selected from non-primed cells, and the lower panel shows the profiles of 40 LOH mutants selected from cells primed with 5 cGy of X-rays. Each column represents a single LOH mutant. The rows represent regions of chromosome 17 diagrammed in the upper right insert. Shaded squares represent deleted regions and hatched squares represent exchanged regions (see text). The region numbers refer to the 11 microsatellite regions: (1) D17S588; (2) D17S1784; (3) D17S785; (4) D17S789; (5) D17S802; (6) D17S807; (7) D17S928; (8) D17S932; (9) D17S1299; (10) D17S1566; (11) THRA (see ref. [29]). The star symbol represents a "small" type 2 hemizygous event in which the deletion is restricted to *TK* locus.

type 2 events in both the non-primed (13 of 15 mutants) and primed (26 of 27 mutants) groups. Small type 2 deletions – those restricted to the *TK* locus (Fig. 2) – were infrequent in both groups (3 of 15 mutants in non-primed cells and 3 of 27 in primed cells). Similarly, the proportion of large deletion mutants (expanding to the region beyond region 8, Fig. 2) was also similar in the primed (18 of 27 mutants) and non-primed (7 of 15 mutants) groups. Homozygous LOH events, on the other hand, which are considered to be the result of homologous recombination (HR) repair of DSBs, were primarily identified as type 1 events in both primed (10 of 13) and non-primed (12 of 13) groups. Interestingly, small homozygous LOH events (where only a single region was replaced, Fig. 2) were recovered from the primed cells (2 of 13 (3 0f 14)), but not from the non-primed cells (0 of 13).

### 3.5. Analysis of non-LOH-mutants

We detected many types of alterations in the non-LOH mutant cDNAs (Table 5). The proportion of single base-substitutions among all the mutations identified as this class was 1/8 (13%) in the primed cells, and this value was clearly lower than 7/18 (87%) in the unprimed cells. G and C bases were targeted in base substitution mutations, except for a single case of an A to T transversion (Table 5). Most (4/5) of the double-base changes consisted of a single base deletion (causing a frameshift) and a base substitution, except for a single case of a GC to TA double transversion in a radioadapted mutant. It is difficult to estimate the effect of priming on the induction of the double-base change events from the limited number of cells involved. Similar difficulties were also found in the other mutational events in this class such as triple-base changes, multiple-base changes and exon skipping. In addition, the proportion of abnormal transcription events (both functional and non-functional *TK* alleles are equally transcribed) was also similar in the radioadapted (1/8, 13%) and the non-adapted (3/18, 17%) group, although its origin was not identified.

## 4. Discussion

The radioadaptation conditions used in this study (5 cGy of priming X-rays followed in 6 h by 2 Gy of challenging X-rays) were similar to those used in other studies [4,6,11,12,14,16]. The *TK* mutation frequency

Table 5
Nature of the isolated non-LOH mutants

| Type of mutation | | Number of identified mutants | | | |
|---|---|---|---|---|---|
| Specific changes | [Position: exon] | Non-primed exposure | | Primed (5 cGy) pre-X-ray | |
| Single base substitutions | | 7 | | 1 | |
| G → A (Gly → Glu) | [56:1] | | 3 | | 0 |
| C → T (Gln → Stop) | [64:1] | | 1 | | 0 |
| C → A (Ser → Stop) | [89:2] | | 1 | | 0 |
| A → T (Ser → Cys) | [97:2] | | 1 | | 0 |
| G → C (Leu → Phe) | [108:2] | | 0 | | 1 |
| G → A (Glu → Lys) | [430:6] | | 1 | | 0 |
| Double base changes | | 3 | | 2 | |
| G → A (Gly → Glu)/Del. C | [56:1/676:7] | | 1 | | 0 |
| G → C (Leu → Phe)/Del. A | [108:2/686:7] | | 0 | | 1 |
| Add. C/Del. G | [232:4/641:7] | | 1 | | 0 |
| GC → TA (Leu Asp → Leu Asn) | [372 and 373:5] | | 0 | | 1 |
| G → A (Glu → Lys)/Del. G | [430:6/447:6] | | 1 | | 0 |
| Triple base changes | | 0 | | 1 | |
| Del. G/C → T (Ile → Ile)/C → T (Leu → Leu) | [92:1/288:4/561:7] | | 0 | | 1 |
| Multiple base changes | | 1 | | 1 | |
| CC → AT (Thr Gln → Thr Stop)/G → A (Gln → Gln)/G → A (Gln → Gln)/Add. C/Del. G | [51 and 52:1/66:1/667:7/232:4/641:7] | | 0 | | 1 |
| Base changes at 20 sites | | | 1 | | 0 |
| Exon skipping, abnormal splicing and deletion | | 3 | | 2 | |
| Del. of a part of exon 1 (48 bases) | [161–209: 1] | | 1 | | 1 |
| Abnormal splicing of intron (between exons 1 and 2) | | | 1 | | 0 |
| Skipping of exon 3 (Del. 111 bases) | [99–203: 3] | | 1 | | 0 |
| Skipping of exon 5 (Del. 90 bases) | [304–393: 5] | | 0 | | 1 |
| Abnormal transcription | | 3 | | 1 | |
| Both functional and non-functional alleles are equally transcribed | | | | | |
| Unidentified | | 1 | | 0 | |
| Total | | 18 | | 8 | |

we observed after the challenge X-rays ($18.3 \times 10^{-6}$) was reduced by the 5-cGy priming exposure to about 62% of the non-primed level ($11.4 \times 10^{-6}$). Taking into consideration the *TK* spontaneous mutation frequency observed in our recent study ($3.0 \times 10^{-6}$) [32], the increase in MF induced by 2 Gy of X-rays was reduced from 6.1-fold to 3.8-fold.

We originally planned this study to determine whether radioadaptation would alter the characteristics of X-ray-induced LOH events. X-ray-induced interstitial deletions are likely to be the result of NHEJ repair of DSBs, and this type of mutation was the one we recovered most frequently after 2 Gy X-ray irradiation in our previous study [24]. We also found that carbon-ion beam irradiation induced interstitial deletions more efficiently than the same dose of X-rays [26,27], which we interpreted as the result of a higher occurrence of inaccurately repaired DSBs. In the present study, however, we found that the frequency of hemizygous LOH mutations, as well as their size and the distribution of deleted regions on chromosome 17, was similar for radioadapted and non-adapted cells. Those results are not consistent with reports suggesting that enhanced repair of DSBs reduces chromosomal alterations [21,22]. An entire genome assay might lead to results similar to the ones in those reports, but our observations were restricted to the *TK* locus on chromosome 17.

On the other hand, we observed a decrease in the induction of homozygous LOH events in the primed cells, which suggests that priming enhanced the HR repair of DSBs. We recently constructed a model system to follow the fate of a single DSB introduced by the restriction enzyme I-sceI at a specific site in the *TK* gene in TK6 cells [31]. In preliminary exper-

iments, low dose/low-dose rate γ-irradiation (30 mGy at 1.2 mGy/h) did not significantly affect end joining (EJ) repair of this specific DSB, but it enhanced the efficiency of HR repair by about 50% (unpublished data). The small homozygous LOH events we observed in the primed cells in the present study (3/14) might reflect this enhanced HR, but we must examine whether the adaptive response was really involved because we also recovered small homozygous LOH mutants after the low-dose/low-dose rate γ-ray exposures [32]. The decrease in the frequency of single-base substitutions that we observed in primed cells (1/48) versus non-primed cells (7/46) (Tables 4 and 5) is rarely influenced by counting base substitutions accompanied by single base deletions (which would result in 2/48 for primed cells and 9/46 for non-primed cells), so the most likely mechanism for the reduced induction of non-LOH mutants was suppression of base substitutions.

One of the possible targets for radioadaptation is oxidative base damage. In fact, down-regulation of the human *CDC16* gene that occurs after oxidative stress causes more rapid and efficient repair in adapted (2 cGy pre-irradiated) human lymphoblastoid cells challenged with 4 Gy irradiation [6]. On the other hand, oxidative base excision repair enzymes, including DNA glycosylases, hOGG1 and hNth1, are reportedly not up-regulated at the post-transcriptional level in γ-ray-primed TK6 cells [33]. Since DNA glycosylase can suppress base substitution, we need to examine whether radioadaptation enhances the enzyme's activity under the present condition. Alternatively, base substitution activity might not accurately reflect DNA glycosylase activity because attempted base excision repair of IR damage by the enzyme can lead to lethal and mutagenic DSBs [34].

A variety of untargeted effects may contribute to the short- and long-term fate of a cell exposed to IR [35]. An example is the possible involvement of a "radioadaptive bystander" effect in human lung fibroblasts [36]. The reduction of radiosensitivity in cells with a wild type *p53* gene by a radiation-induced, nitric oxide (NO)-mediated bystander effects may be a manifestation of the radioadaptive response [37,38]. This possibility is supported by the finding that the NO-induced apoptosis observed in lymphoblastoid and fibroblast cells depends on the phosphorylation and activation of p53 [39]. However, it is still unclear whether the NO-mediated pathway also contributes to the mutagenic adaptation. The de novo protein synthesis is required for expression of adaptive responses [22,40], and gene expression studies are improving our understanding of the molecular mechanisms underlying the radioadaptive response [9,10,40,41]. Our laboratory is also focusing on the molecular mechanisms involved in radioadaptation, especially the expression of genes involved in DNA base and nucleotide excision repair.

## Acknowledgements

This study was partially supported by the Budget for Nuclear Research of the Ministry of Education, Culture, Sports, Science and Technology, and was reviewed by the Atomic Energy Commission of Japan. We thank Dr. Miriam Bloom (SciWrite Biomedical Writing & Editing Services) for professional editing.

## References

[1] G. Olivieri, Y. Bodycote, S. Wolf, Adaptive response of human lymphocytes to low concentrations of radioactive thymidine, Science 223 (1984) 594–597.

[2] S. Wolf, Aspects of the adaptive response to very low doses of radiation and other agents, Mutat. Res. 358 (1996) 135–142.

[3] S. Wolf, The adaptive response in radiobiology: evolving insights and implications, Environ. Health Pesrpect. 106 (1998) 277–283.

[4] O. Rigaud, E. Moustacchi, Radioadaptation for gene mutation and the possible molecular mechanisms of the adaptive response, Mutat. Res. 358 (1996) 127–134.

[5] M. Wojewodska, M. Kruzewski, K. Iwanenko, I. Szumiel, Effects of signal transduction in adapted lymphocytes: micronuclei frequency and DNA repair, Int. J. Radiat. Biol. 71 (1997) 245–252.

[6] P.-K. Zhou, O. Rigaud, Down-regulation of the human *CDC16* gene after exposure to ionizing radiation: a possible role in the radioadaptive response, Radiat. Res. 155 (2001) 43–49.

[7] M.S. Sasaki, Y. Ejima, A. Tachibana, T. Yamada, K. Ishizaki, T. Shimizu, T. Nomura, DNA damage response pathway in radioadaptive response, Mutat. Res. 504 (2002) 101–118.

[8] I. Szumiel, Adaptive responses: stimulated DNA repair or decreased damage fixation? Int. J. Radiat. Biol. 81 (2005) 233–241.

[9] M.A. Coleman, E. Yin, L.E. Peterson, D. Nelson, K. Sorensen, J.D. Tucker, A.J. Wyrobeck, Low-dose irradiation alters the transcript profiles of human lymphoblastoid cells inducing genes associated with radioadaptive response, Radiat. Res. 164 (2005) 369–382.

[10] H.P. Wang, X.H. Long, Z.Z. Sun, O. Rigaud, Q.Z. Xu, Y.C. Huang, J.L. Sui, B. Bai, P.K. Zhou, Identification of differentially transcribed genes in human lymphoblastoid cells irradiated with 0.5 Gy of γ-ray and the involvement of low dose radiation inducible CHD6 gene in cell proliferation and radiosensitivity, Int. J. Radiat. Biol. 82 (2006) 181–190.

[11] S.G. Swant, G. Randers-Pehrson, N.F. Metting, E.J. Hall, Adaptive response and the bystander effect induced by radiation in C3H 10T1/2 cells in culture, Radiat. Res. 156 (2001) 177–180.

[12] H.N. Zhou, G. Randers-Pehrson, C.R. Geard, D.J. Brenner, E.J. Hall, T.K. Hei, Interaction between radiation-induced adaptive response and bystander mutagenesis in mammalian cells, Radiat. Res. 160 (2003) 512–516.

[13] W.M. Bonner, Thresholds, bystander effect, and adaptive response, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 4973–4975.

[14] S.A. Mitchell, S.A. Marino, D.J. Brenner, E.J. Hall, Bystander effect and adaptive response in C3H 10T(1/2) cells, Int. J. Radiat. Biol. 80 (2004) 465–472.

[15] T.K. Hei, R. Persaud, H. Zhou, M. Suzuki, Genotoxicity in the eyes of bystander cells, Mutat. Res. 568 (2004) 111–120.

[16] T. Ikushima, Chromosomal response to ionizing radiation reminiscent of an adaptive response in cultured Chinese hamster cells, Mutat. Res. 180 (1987) 215–221.

[17] B.J.S. Sanderson, A.A. Morely, Exposure of human lymphocytes to ionizing radiation reduces mutagenicity by subsequent radiation, Mutat. Res. 164 (1986) 151–347.

[18] P.K. Zhou, X.Y. Liu, W.Z. Sun, Y.P. Zhang, Y.P.K. Wei, Cultured mouse SR-1 cells exposed to low-dose of γ-rays become less susceptible to the induction of mutations by radiation as well as bleomycin, Mutagenesis 8 (1993) 109–111.

[19] A.M. Ueno, D.B. Vannais, S.L. Gustafson, J.C. Wong, C.A. Waldren, A low adaptive dose of gamma-rays reduced the number and altered the spectrum of S1 mutants in human hamster hybrid cells, Mutat. Res. 358 (1996) 161–169.

[20] E.I. Azzam, G.P. Raaphorst, R.E. Mitchel, Radiation-induced adaptive response for protection against micronucleus formation and neoplastic transformation in C3H 10T1/2 mouse embryo cells, Radiat. Res. 138 (1994) S28–S31.

[21] O. Rigaud, D. Papadopoulo, E. Moustacchi, Decreased deletion mutation in radioadapted human lymphoblast, Radiat. Res. 133 (1993) 94–101.

[22] T. Ikushima, H. Aritomi, J. Morisita, Radioadaptive response: efficient repair of radiation-induced DNA damage in adapted cells, Mutat. Res. 358 (1996) 193–198.

[23] T.R. Skopek, H.L. Liber, B.W. Penman, W.G. Thilly, Isolation of a human lymphoblastoid line heterozygous at the thymidine kinase locus: possibility for a rapid human cell mutation assay, Biochem. Biophys. Res. Commun. 84 (1978) 411–416.

[24] M. Honma, M. Hayashi, T. Sofuni, Cytotoxic and mutagenic responses to X-rays and chemical mutagens in normal and p53-mutated human lymphoblastoid cell, Mutat. Res. 374 (1996) 89–98.

[25] M. Honma, L.S. Zhang, M. Hayashi, K. Takeshita, Y. Nakagawa, N. Tanaka, T. Sofuni, Illegitimate recombination leading to allelic loss and unbalanced translocation in p53-mutated human lymphoblastoid cells, Mol. Cell. Biol. 17 (1997) 4774–4781.

[26] S. Morimoto, T. Kato, M. Honma, M. Hayashi, F. Hanaoka, F. Yatagai, Detection of genetic alterations induced by low-dose X rays: analysis of loss of heterozygosity for *TK* mutation in human lymphoblastoid cells, Radiat. Res. 157 (2002) 533–538.

[27] S. Morimoto, M. Honma, F. Yatagai, Sensitive detection of LOH events in a human cell line after C-ion beam exposure, J. Radiat. Res. 43 (Suppl.) (2002) S163–S167.

[28] Y. Umebayashi, M. Honma, T. Abe, H. Ryuto, H. Suzuki, T. Shimazu, N. Ishioka, M. Iwaki, F. Yatagai, Mutation induction after low-dose carbon-ion beam irradiation of frozen human cultured cells, Biol. Sci. Space 19 (2005) 237–241.

[29] F. Yatagai, S. Morimoto, T. Kato, M. Honma, Further characterization of loss of heterozygosity enhanced by p53 abrogation in human lymphoblastoid TK6 cells: disappearance of endpoint hotspots, Mutat. Res. 560 (2004) 133–145.

[30] A.J. Grosvsky, B.N. Walter, C.R. Giver, DNA-sequence specificity of mutations at the human thymidine kinase locus, Mutat. Res. 289 (1993) 231–243.

[31] M. Honma, M. Izumi, M. Sakuraba, S. Tadokoro, H. Sakamoto, W. Wang, F. Yatagai, M. Hayashi, Deletion, rearrangement, and gene conversion; genetic consequences of chromosomal double-strand breaks in human cells, Environ. Mol. Mutagen. 42 (2003) 288–298.

[32] Y. Umebayashi, M. Honma, M. Suzuki, H. Suzuki, T. Shimazu, N. Ishioka, M. Iwaki, F. Yatagai, Mutation induction in cultured human cells after low-dose and low-dose-rate γ-ray irradiation: detection by LOH analysis, J. Radiat. Res. 48 (2006) 7–11.

[33] M. Inoue, G.-P. Shen, M.A. Chaudhry, H. Galick, J.O. Blaisdell, S.S. Wallace, Expression of the oxidative base excision repair enzymes is not induced in TK6 human lymphoblastoid cells after low doses of ionizing radiation, Radiat. Res. 161 (2004) 409–417.

[34] N. Yang, H. Galick, S.S. Wallace, Attempted base excision repair of ionizing radiation damage in human lymphoblastoid cells produces lethal and mutagenic double-strand breaks, DNA Repair 3 (2004) 1323–1334.

[35] P.J. Coates, S.A. Lorimore, E.G. Wright, Damaging and protective cell signaling in the untargeted effects of ionizing radiation, Mutat. Res. 568 (2004) 5–20.

[36] R. Iyer, B.E. Lehnert, Low-dose, low-LET ionizing radiation-induced radioadaptation and associated early responses in unirradiated cells, Mutat. Res. 503 (2002) 1–9.

[37] H. Matsumoto, A. Takahashi, T. Ohnishi, Radiation-induced adaptive and bystander effects, Biol. Sci. Space 18 (2004) 247–254.

[38] H. Matsumoto, A. Takahashi, T. Ohnishi, Nitric oxide radicals choreograph a radioadaptive response, Cancer Res. 67 (2007) 8574–8579.

[39] L.M. McLaughlin, B. Demple, Nitric oxide-induced apoptosis in lymphoblastoid and fibroblast cells dependent on the phosphorylation and activation of p53, Cancer Res. 65 (2005) 6097–6104.

[40] J.H. Yongblom, J.K. Wiencke, S. Wolf, Inhibition of the adaptive response of human lymphocytes to very low doses of ionizing radiation by the protein synthesis inhibitor cycloheximide, Mutat. Res. 227 (1989) 257–261.

[41] L.-H. Ding, M. Shingyoji, F. Chen, J.-J. Hwang, S. Burma, C. Lee, J.-F. Chen, D.J. Chen, Gene expression profiles of normal human fibroblasts after exposure to ionizing radiation: a comparative study of low and high doses, Radiat. Res. 164 (2005) 17–26.

doi:10.1016/j.jmb.2008.01.033 J. Mol. Biol. (2008) 377, 1015–1023

Available online at www.sciencedirect.com

ScienceDirect

# Miscoding Properties of 2′-Deoxyinosine, a Nitric Oxide-Derived DNA Adduct, during Translesion Synthesis Catalyzed by Human DNA Polymerases

**Manabu Yasui[1]*, Emi Suenaga[2], Naoki Koyama[1], Chikahide Masutani[3], Fumio Hanaoka[3], Petr Gruz[1], Shinya Shibutani[4], Takehiko Nohmi[1], Makoto Hayashi[1] and Masamitsu Honma[1]**

[1]*Division of Genetics and Mutagenesis, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya, Tokyo 158-8501, Japan*

[2]*Division of Pharmacognosy, Phytochemistry and Narcotics, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya, Tokyo 158-8501, Japan*

[3]*Cellular Biology Laboratory, Graduate School of Frontier Biosciences, Osaka University, 1-3 Yamada-oka, Suita, Osaka 565-0871, Japan*

[4]*Department of Pharmacological Sciences, State University of New York at Stony Brook, Stony Brook, NY 11794-8651, USA*

*Received 26 November 2007; received in revised form 10 January 2008; accepted 14 January 2008*
Available online 18 January 2008

***Edited by J. Karn***

Chronic inflammation involving constant generation of nitric oxide (˙NO) by macrophages has been recognized as a factor related to carcinogenesis. At the site of inflammation, nitrosatively deaminated DNA adducts such as 2′-deoxyinosine (dI) and 2′-deoxyxanthosine are primarily formed by ˙NO and may be associated with the development of cancer. In this study, we explored the miscoding properties of the dI lesion generated by Y-family DNA polymerases (pols) using a new fluorescent method for analyzing translesion synthesis. An oligodeoxynucleotide containing a single dI lesion was used as a template in primer extension reaction catalyzed by human DNA pols to explore the miscoding potential of the dI adduct. Primer extension reaction catalyzed by pol α was slightly retarded prior to the dI adduct site; most of the primers were extended past the lesion. Pol η and pol κΔC (a truncated form of pol κ) readily bypassed the dI lesion. The fully extended products were analyzed by using two-phased PAGE to quantify the miscoding frequency and specificity occurring at the lesion site. All pols, that is, pol α, pol η, and pol κΔC, promoted preferential incorporation of 2′-deoxycytidine monophosphate (dCMP), the wrong base, opposite the dI lesion. Surprisingly, no incorporation of 2′-deoxythymidine monophosphate, the correct base, was observed opposite the lesion. Steady-state kinetic studies with pol α, pol η, and pol κΔC indicated that dCMP was preferentially incorporated opposite the dI lesion. These pols bypassed the lesion by incorporating dCMP opposite the lesion and extended past the lesion. These relative bypass frequencies past the dC:dI pair were at least 3 orders of magnitude higher than those for the dT:dI pair. Thus, the dI adduct is a highly miscoding lesion capable of generating A→G transition. This ˙NO-induced adduct may play an important role in initiating inflammation-driven carcinogenesis.

© 2008 Elsevier Ltd. All rights reserved.

*Keywords:* inflammation; nitric oxide; DNA adduct; translesion synthesis; nonradioactive analysis

*Corresponding author. E-mail address: m-yasui@nihs.go.jp

Abbreviations used: ˙NO, nitric oxide; dI, 2′-deoxyinosine; dX, 2′-deoxyxanthosine; dA, 2′-deoxyadenosine; 8-OxodG, 8-oxo-2′-deoxyguanosine; dNTP, 2′-deoxynucleoside triphosphate; Alexa546, Alexa Fluor 546 dye; pol α, human DNA polymerase α; pol η, human DNA polymerase η; pol κ, human DNA polymerase κ; pol κΔC, a truncated form of pol κ; $F_{ins}$, frequency of insertion; $F_{ext}$, frequency of extension; 8-$NO_2$-dG, 8-nitro-2′-deoxyguanosine; dCMP, 2′-deoxycytidine monophosphate; dTMP, 2′-deoxythymidine monophosphate; dTTP, 2′-deoxythymidine triphosphate; dCTP, 2′-deoxycytidine triphosphate; dGTP, 2′-deoxyguanosine triphosphate; Cy3, Cyanin 3; endo V, endonuclease V.

0022-2836/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.

## Introduction

Chronic inflammation involving constant generation of nitric oxide ($^{\cdot}NO$) by macrophages has been recognized as a factor related to carcinogenesis.[1-3] $^{\cdot}NO$ can attack neighboring epithelial and stromal cells by damaging the DNA, altering their genome stability. There are two possible major pathways for the $^{\cdot}NO$ reaction (Fig. 1). One pathway involves the combination of $^{\cdot}NO$ and superoxide with the formation of highly toxic peroxynitrite ($ONOO^-$). Spontaneous hydrolysis of peroxynitrite under physiological conditions generates secondary radical species ($^{\cdot}NO_2$, $^{\cdot}OH$, and $CO_3^{\cdot -}$) that induce oxidation and nitration of diverse DNA adducts[4,5] such as 8-oxo-2'-deoxyguanosine (8-OxodG) and 8-nitro-2'-deoxyguanosine (8-$NO_2$-dG).[6,7] A second involves nitrosative deamination of DNA base by $^{\cdot}NO$ via formation of several nitrosating agents, which predominantly exist as nitrous anhydride ($N_2O_3$) at physiological pH.[8] The DNA base products by nitrosative deamination mainly involve conversion of adenine to hypoxanthine [2'-deoxyinosine (dI)] (Fig. 2), guanine to xanthine [2'-deoxyxanthosine (dX)], and cytosine to uracil.[9-12]

The spectrum of nitrosative DNA adducts in $N_2O_3$-treated plasmid DNA was composed of approximately 2% dG–dG cross-links, 4–6% abasic sites, and 25–35% each of dI, dX, and 2'-deoxyuracyl.[12,13] Moreover, dI and dX, as well as lipid peroxidated adducts, were increased in the cellular DNA of tissues from the $^{\cdot}NO$-overproducing SJL mouse model of inflammation.[14] The increased production of $^{\cdot}NO$ was associated with an increased mutation frequency.[15] When human TK6 cells were exposed to $^{\cdot}NO$, the increase in mutation rates observed at hypoxanthineguanine phosphoribosyltransferase and thymidine kinase gene loci correlated with the 40-fold increase of dI and dX more than did that of the controls in the cellular DNA.[10] Thus, the dI adduct is one of the major $^{\cdot}NO$-derived DNA lesions and may contribute to the burden of carcinogenesis in inflammation tissue.

2'-deoxyadenosine (dA) 2'-deoxyinosine (dI)

Fig. 2. Structures of dA and dI adducts.

Site-specifically dI-modified oligodeoxynucleotides have been used as DNA templates for investigating the miscoding events using only mouse pol α and rat pol β.[16] The previous report showed that rat pol β inserted only 2'-deoxycytidine monophosphate (dCMP) opposite the dI lesion and that mouse pol α tended to incorporate dCMP and 2'-deoxythymidine monophosphate (dTMP) opposite the lesion. However, the miscoding events were investigated with the detection of mismatched base pairs by loss of a restriction enzyme recognition site. No quantitative analysis, therefore, has been performed for determination of miscoding events generated by dI.

Human DNA pol η[17] and pol κ[18,19] that are associated with translesion synthesis past a variety of DNA lesions[20,21] were examined. We have here explored the miscoding properties of the dI lesion that occurred during DNA replication catalyzed by

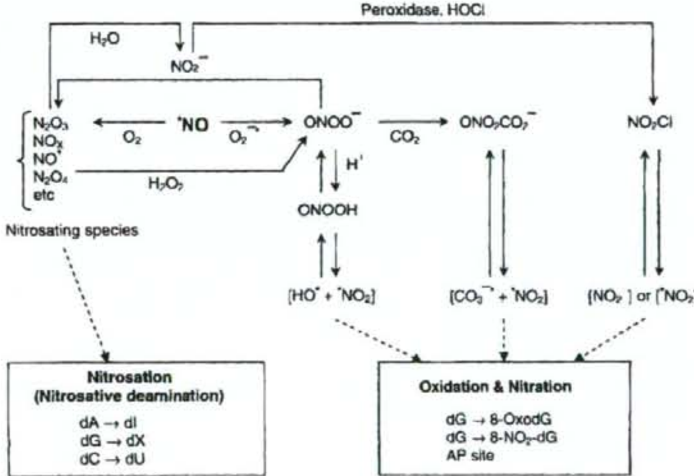



Fig. 1. Possible pathways for the formation of $^{\cdot}NO$-induced DNA adducts.