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Fundamental and Molecular Mechanisms of Mutagenesis

Induction of chromosome aberrations in Syrian hamster renal cortical cells by various estrogens

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Abstract

Estrogens, both natural and synthetic, have been implicated in carcinogenesis at different organ sites in a variety of animals, including man, for more than six decades. However, the molecular mechanism(s) involved in the carcinogenic action of estrogens still remains both controversial and elusive. Cytogenetic damage in the hamster kidney has been studied after in vivo treatment with either potent or weak estrogens for varying periods. Compared to age-matched untreated controls, diethylstilbestrol (DES) treatment resulted in significant increases in the number of chromatid gaps and breaks, chromosome breaks, and endoreduplicated cells in hamster renal cortical cells. These chromosomal aberrations (CA) were cumulative with continued hormone exposure from 1.0 to 5.0 months. However, chromosome exchanges as a result of the breaks were not elevated. After 5.0 months of hormone treatment, potent estrogens such as 17β-estradiol and Moxestrol exhibited similar frequencies of CA in the hamster kidney to that found for DES, whereas weak estrogens such as 17α-estradiol and β-dienestrol exhibited CA frequencies that were not significantly different from untreated levels. Ethinylestradiol treatment for a similar period resulted in significant increases in chromatid gaps, although these did not evolve into increases in either chromatid or chromosome breaks, and in a rise in endoreduplicated cells. These results raise the possibility that the CA generated after estrogen treatment may be involved in renal tumorigenic processes.

Keywords: Oestrogen induction; Chromosome aberrations; Hamster kidney; Tumorigenesis

1. Introduction

Estrogens have been implicated in carcinogenesis in numerous animals, including man (Li and Li, 1994a; Henderson et al., 1982). The molecular mechanism(s) involved in the carcinogenic action of estrogens remains both controversial and elusive. Recent evidence, however, from both in vivo and in vitro studies strongly suggests that estrogens are epi-genotoxic carcinogens (Li and Li, 1994b; Li et al., 1993; Barrett et al., 1987); that is, they do not behave as direct acting mutagenic or DNA-damaging agents via covalent interaction with genetic material but instead elicit heritable changes by alternative mechanisms. The end result is ultimately to cause heritable changes in the structure or sequence of the genetic material at

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the level of the nucleic acids, genes, or chromosomes. While it has been shown that both natural and synthetic estrogens are capable of inducing unscheduled DNA synthesis (UDS) in HeLa cells, mouse germ cells, and Syrian hamster embryo (SHE) cells (Martin et al., 1978; Racine and Schmid, 1983; Tsutsui et al., 1984), and sister-chromatic exchange (SCE) in rat and human hepatoma cell lines (Buenaventura et al., 1984), Chinese hamster ovary (CHO) cells (Kochhar, 1985), and fibroblasts and human lymphocytes (Rüdiger et al., 1979; Hill and Wolff, 1983), these observations have largely been demonstrated in vitro in either nonepithelial or transformed cells exposed to estrogens. However, it has recently been shown that both diethylstilbestrol (DES) and 17β-estradiol (17β-E_2) induced SCE in cultured neonatal mouse uterine cervical epithelium (Hillbertz-Nilsson and Forsberg, 1989). In contrast, numerous earlier reports have presented conflicting data indicating that the effect of estrogen treatment in the induction of chromosomal aberrations (CA) in various in vitro systems was either negligible or absent (Banduhn and Obe, 1985; Mehnert et al., 1985; Husum et al., 1982; Drevon et al., 1981; Chrisman and Baumgartner, 1981).

Chronic exposure of male but not female Syrian hamsters to either steroidal or stilbene estrogens results in essentially 100% incidence of multiple bilateral renal neoplasms (Kirkman, 1959; Li and Li, 1987). Evidence has accumulated which strongly indicates that the estrogen-induced renal tumor arises from undifferentiated committed epithelial stem cells in the interstitium (Llombart-Bosch and Peydro 1975; Gonzalez et al., 1989; Oberley et al., 1991). Not all estrogens are equally active in inducing these renal tumors (Li and Li, 1987). With the exception of ethinylestradiol (EE), which elicits only a 10% renal tumor incidence, potent estrogens (17β-E_2, DES, hexestrol, and 11β-methoxyethinylestradiol (Mox)) exhibit high incidences of renal neoplasms compared to weak estrogens (estriol, 4-hydroxyestrone). Moreover, estrogens which possess low or negligible estrogenic activity (17α-E_2, β-dienestrol, 2-hydroxyestradiol) do not induce kidney tumors. The lack of strong carcinogenic activity of EE in the hamster kidney, despite its potent estrogenic activity, is evidently the result of its unique and consistent effects on the proliferation of a subset of renal tubule cells which are unaffected by other potent estrogens found to be highly carcinogenic in this model (Oberley et al., 1991).

The present study is the first report that evaluates the cytogenetic damage induced in hamster renal tubules after progressive in vivo DES treatment, and the prolonged effect of either potent or weak estrogens on CA induction.

2. Materials and methods

Animals and treatment

Adult male noninbred castrated Syrian hamsters weighing 80–90 g were purchased from Harian Sprague Dawley, Indianapolis, IN. Hamsters were housed in facilities certified by the American Association for the Accreditation of Laboratory Animal Care. They were maintained on a 12-h light: 12-h dark cycle, fed certified rodent chow (Ralston-Purina 5002), and given tap water ad libitum. The animal studies were carried out in adherence to the guidelines established in the 'Guide for the Care and Use of Laboratory Animals', U.S. Department of Health and Human Resources (NIH, 1985). Groups of animals (five per group) were implanted subcutaneously, in the shoulder region, with 20-mg pellets of DES, 17β-E_2, Mox, EE, 17α-E_2, or β-dienestrol for varying intervals as appropriately indicated. The hormone pellets were prepared, without binder, by Hormone Pellet Press, Westwood, KS. In order to maintain constant hormonal levels, additional pellets were implanted every 2.5 months. The mean daily absorption expressed in μg for the estrogen used was as follows: DES, 139.8 ± 12.8; 17β-E_2, 125.2 ± 4.0; Mox, 120.5 ± 4.0; 17α-E_2, 103.7 ± 2.0; EE, 184.7 ± 8.0; and β-dienestrol, 94.3 ± 2.0. Age- and weight-matched hamsters (sham-operated) were used as controls, receiving the same diet but no hormone implants.

Isolation and culture of renal cortical cells

Animals were killed by decapitation. Renal cortical cells were isolated and cultured accord-

ing to the method of Kovacs and Frisch (1989) and Oberley et al. (1989) with slight modification. Briefly, whole kidneys were removed and cortices and medullas separated. The cortical tissue was transferred into sterile Dounce tissue homogenizers containing serum-free supplemented Waymouth MB 752/1 medium (SFSW) (Gibco BRL, Grand Island, NY) (Oberley et al., 1989) and fitted with loose pestles. The tissues were then subjected to 9–10 strokes. Intact cortical cells and fragments were separated and washed thoroughly with 10 mM phosphate buffer saline solution (PBS), pH 7.2. Cells were transferred into T-flasks containing 10 ml serum-free medium and collagenase (250 U/ml, Sigma Chemical Co., St. Louis, MO) and incubated at 37°C for 3 min. After the incubation, the cortical tissue cells and tubule fragments were washed with 30 ml of Waymouth, centrifuged at $800 \times g$ and then uniformly suspended in 3.0 ml of SFSW medium. The tubule fragments were cultured on MDCK-basement membrane coated petri dishes, which were prepared by the method of Yang et al. (1987). The small clusters of cortical proximal tubule fragments were cultured in the same chemically defined serum-free medium previously described. Depending on the rate of cell proliferation, cytogenetic analyses were carried out after 2–4 days of culture after an initial attachment period of 3–5 days.

Preparation and analysis of chromosomes

For metaphase chromosomes, cortical cell cultures were treated with colchicine (0.4 μg/ml) for 3–4 h. The cortical cells were then trypsinized and treated with hypotonic solution (0.075 M

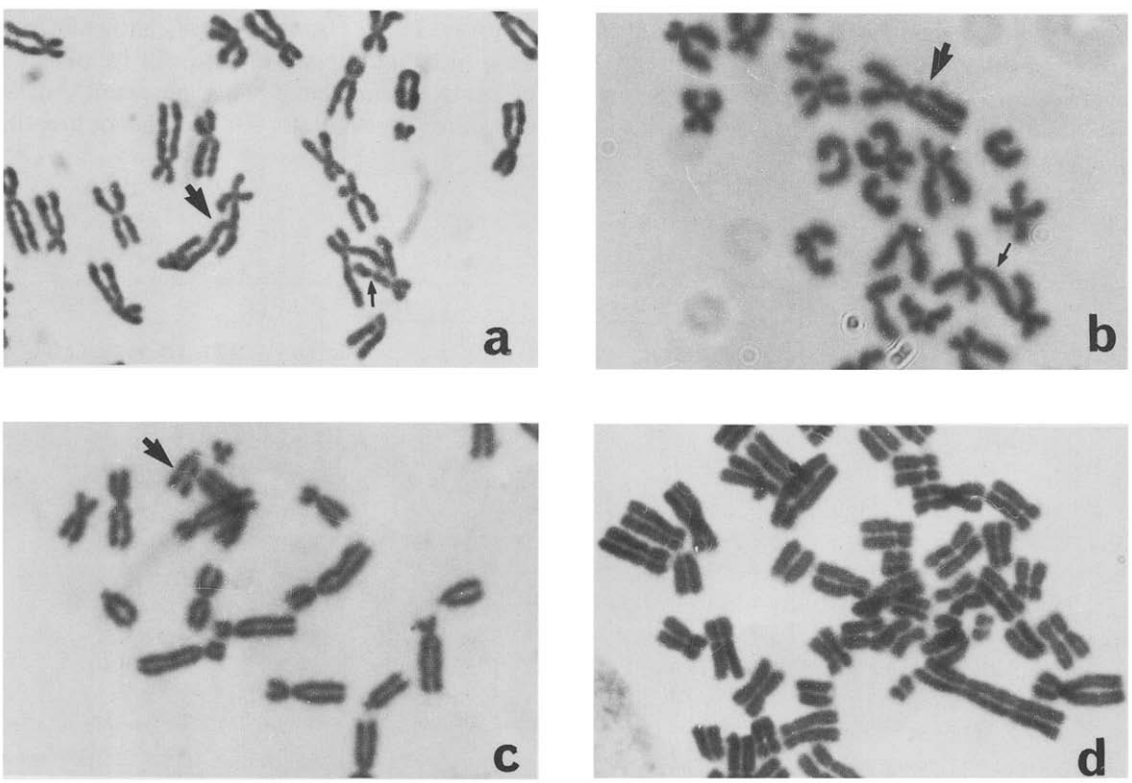

Fig. 1. Examples of identified metaphase spreads exhibiting chromosomal aberrations induced after 5.0 months of DES treatment in Syrian hamster kidney proximal tubule cells. (a) Chromatid gap (small arrow) and chromatid break with exchanges (large arrow). (b) Chromatid break (large arrow) and chromatid exchange (small arrow). (c) Isochromatic/chromosome break. (d) Endoreduplication. (Magnification, 1500 × .)

KCl) at 37°C for 30 min. The cell suspensions were centrifuged and the pellets fixed in cold acetic acid:methanol (1:3) solution. Slides were prepared by standard air drying method and stained with 2% Giemsa solution (Worton and Duff, 1979). CA were scored by analyzing at least 200 well spread metaphases with 44 ± 2 chromosomes for gaps, chromatid and chromosome breaks and exchanges, and association (Carrano and Natarajan, 1971). Chromatid and chromosome aberrations were scored separately, and the total percentage of CA/cell was subjected to statistical analysis (Sharief et al., 1986). Gaps were recorded but not included in the total frequency of CA. Endoreduplication (endomitosis) was estimated from at least 500 cells/animal and expressed as a percentage.

Statistical analyses

ANOVA analysis was applied to assess the effects of various regimens. The significance of differences in the frequency of aberrations between treated and untreated groups was determined by Student's *t*-test.

3. Results

CA in the renal cortical cells after DES treatment

Fig. 1a–d depicts the CA, including gaps, chromatid and chromosome breaks, and endoreduplications, observed in hamster renal cortical epithelium following 5.0-month DES treatment. There were no significant differences in the frequency of either chromosome lesions (gaps) or chromatid or chromosome aberrations with increasing age corresponding to the time intervals of the estrogen-treated groups (Table 1). DES treatment induced marked increases in CA in renal cortical cells as early as 15 days after hormone treatment (data not shown). Continued DES treatment resulted in significant increases in chromatid gaps (Fig. 1a), breaks (Fig. 1b), and exchanges (Fig. 1a), as well as chromosome breaks (Fig. 1c) and endoreduplicated cells (Fig. 1d) in hamster renal cortical cells compared to untreated age-matched controls. These CA were significantly elevated at each monthly interval of estrogen treatment and evidently accumulated with increasing hormone exposure from 1.0 to 5.0 months of treatment

Table 1
Chromosomal aberrations induced by DES in Syrian hamster kidney proximal tubules

Duration of treatment (months) [a]	Gaps/100 cells [b]		Aberrations/100 cells				% Aberrant cells (mean ± SEM)	% Endoreduplicated cells (mean ± SEM)
	Chromatid	Isochromatid	Chromatid		Chromosome			
			Breaks	Exchanges	Breaks	Exchanges		
Untreated								
0.5	0.3	0	0.2	nd [c]	nd	nd	0.2 ± 0.1	0
1.0	0.4	0	0.3	nd	nd	nd	0.3 ± 0.2	0
2.0	0.2	0	0.2	nd	nd	nd	0.3 ± 0.1	0
3.0	0.3	0.1	0.5	nd	0.1	nd	0.5 ± 0.3	0
4.0	0.5	0	0.5	nd	nd	0.1	0.5 ± 0.2	0
5.0	0.4	0.2	0.4	nd	0.1	nd	0.4 ± 0.2	0
DES-treated								
0.5	3.3	0	4.0	2.1	0.5	nd	5.5 ± 0.5 *	1.2 ± 0.5
1.0	6.1	0.1	8.7	1.0	2.1	nd	9.5 ± 1.7 *	1.3 ± 0.8
2.0	6.6	0	7.0	1.3	2.0	0.1	9.7 ± 1.8 *	4.5 ± 2.0
3.0	8.4	0.3	9.2	1.5	3.6	nd	13.4 ± 1.8 *	5.3 ± 2.1
4.0	9.4	0.5	10.0	4.8	4.3	0.3	15.7 ± 1.4 *	8.0 ± 3.4
5.0	11.7	0.7	11.0	3.9	5.4	0.2	18.3 ± 1.2 *	8.3 ± 2.1

[a] Groups of 5–7 hamsters were used for each group. At least 200 well spread metaphases were analyzed from each animal.
[b] Chromatid and isochromatid gaps were not included as aberrations.
[c] nd, not detected.
* Statistically significant, $p < 0.001$, compared to age-matched untreated controls.

(Table 1). However, chromosome exchanges as a result of the breaks were not elevated. The total number of aberrant cells in the hamster kidney was cumulative, attaining a maximal level at 5.0 months of continuous hormone treatment.

Frequency of CA in hamster renal cortical cells after treatment with different estrogens

Table 2 summarizes the frequency of CA in hamster proximal renal tubule cells after 5.0 months of treatment with both potent and weak estrogens. Potent estrogens such as 17β-E_2 and Mox exhibited a frequency of chromatid gaps, breaks and exchanges, as well as chromosome breaks and endoreduplicated cells, similar to that found for DES. In contrast, weak estrogens such as 17α-E_2 and β-dienestrol, an inactive metabolite of DES, exhibited CA frequencies in the hamster kidney that were not significantly different from untreated levels. Although EE treatment resulted in an appreciable increase in chromatid gaps, these did not evolve into a significant rise in either chromatid or chromosome breaks, or in endoreduplicated cells (Table 2). Although EE treatment resulted in a modest but significant increase in the frequency of aberrant cells in hamster renal cortical cells, the level was approximately fourfold lower than the frequency observed for the other potent estrogens examined. Neither progesterone nor androgen treatment, for the same 5.0-month period, was found to induce any CA in the hamster kidney, indicating that the CA generated by estrogens are hormone specific (data not shown).

4. Discussion

It has been a prevailing notion that CA are the result of direct DNA damage which may lead to mutational events. However, while this may be the case for many chemical agents, alternative explanations have been suggested that may assist in resolving some of the highly conflicting data reported for estrogens by the different groups cited earlier.

In studies of SCE induction in vitro, some nonmutagenic agents such as saccharin, TPA, and sodium bisulfate have been found to produce increases in the frequency of this CA in different cell systems (Kinsella and Radman, 1978; Wolff and Rodin, 1978; Popescu and Di Paolo, 1988). It has been suggested by a number of investigators that such nonmutagenic agents elicit their effects on chromosomes in the absence of significant DNA damage, by promoting either an imbalance of DNA and protein synthesis (Schimke et al., 1986; Van Den Berghe, 1987) or the release of lysosomal or other DNases into the nucleus of damaged cells (Bradley et al., 1987).

Table 2
Chromosomal aberrations induced by different estrogens in Syrian hamster kidney proximal tubules

Estrogen treatment [a]	Gaps/100 cells [b]		Aberrations/100 cells				% Aberrant cells (mean ± SEM)	% Endoreduplicated cells (mean ± SEM)
	Chromatid	Isochromatid	Chromatid		Chromosome			
			Breaks	Exchanges	Breaks	Exchanges		
Untreated	0.4	0.2	0.4	nd [c]	0.1	nd	0.4 ± 0.2	0
DES	11.7	0.7	11.4	3.9	5.4	0.2	18.3 ± 1.2 *	8.3 ± 2.1
17β-Estradiol	9.0	0.4	11.0	3.2	4.0	0.3	15.4 ± 1.0 *	8.5 ± 3.6
Moxestrol	8.3	0.6	10.8	2.8	4.4	1.0	14.8 ± 3.9 *	9.1 ± 3.5
Ethinylestradiol	8.4	0.8	3.4	0.4	0.6	nd	4.1 ± 1.8 *	0
17α-Estradiol	0.5	0	0.6	nd	nd	nd	0.8 ± 0.4	0
β-Dienestrol	2.5	0	2.1	nd	0.6	nd	2.5 ± 1.4	0

[a] Groups 5–7 hamsters were treated for 5.0 months. Age-matched controls were used for the untreated group. At least 200 well spread metaphases were analyzed from each animal.
[b] Chromatid and isochromatid gaps were not included as aberrations.
[c] nd, not detected.
* Statistically significant, $p < 0.001$, compared to age-matched untreated controls.

The role of estrogen metabolites in eliciting in vitro SCE has not been consistent. For example, the combination of DES with either S9, an Aroclor 1254-induced rat liver homogenate, or peroxidase/H_2O did not increase SCE rates in various cell line systems and human lymphocytes (Mehnert et al., 1985). In contrast, other studies suggest that metabolic activation of DES may be necessary for SCE and UDS in other cell systems (Buenaventura et al., 1984; Tsutsui et al., 1984). The in vivo data presented herein are consistent with the notion that metabolic activation is not essential for CA generation since DES and 17β-E_2 are actively metabolized in the hamster kidney, whereas Mox, an equally potent carcinogenic estrogen in this system, is only poorly metabolized (Li and Li, 1992) and yet is able to produce the same percentage of aberrant cells and similar kinds of CA. On the other hand, relatively impotent and noncarcinogenic estrogens exhibit low percentages of aberrant cells and low frequencies of CA. The potent estrogen, EE, which does not have as marked an effect on the same proximal tubule cells as DES or 17β-E_2 (Oberley et al., 1991), produces the same frequency of chromatid and isochromatid gaps as these latter estrogens, but EE treatment does not result in a similar frequency of chromatid and chromosome breaks and endoreduplicated cells. It should be noted that these estrogen-specific CA were not detected following in vitro addition of any of the estrogens tested at concentration ranges of 1–10 nM in cultured hamster proximal tubule cells (Banerjee, Li and Li, unpublished data).

It has been clearly demonstrated that estrogens have a colcemid-like effect on various cell systems and that estrogens, particularly DES, may act as a mitotic poison affecting the spindle apparatus (Sawada and Ishidate, 1978). Since colcemid itself is capable of producing CA (Satya-Prakash et al., 1986), interference with mitotic activity or cell cycle progression other than direct damage to DNA remains a distinct possibility for estrogens. Consistent with this notion is the high incidence of endoreduplication seen after treatment with potent carcinogenic estrogens, which suggests that a block at the G2 phase of the cell cycle may allow some cells to return to the G1 phase without undergoing mitosis (Sutou and Arai, 1975).

The relationship between the observations presented herein and renal tumorigenesis induced by estrogens in the hamster has yet to be resolved despite the intriguing finding that carcinogenic estrogens generate a higher frequency of CA in the kidney compared to noncarcinogenic estrogens. To further elucidate the significance of these CA elicited by carcinogenic estrogens during the renal tumorigenic process, it will be necessary to ascertain whether these CA are nonrandom and stable in nature.

References

Banduhn, and G. Obe (1985) Mutagenicity of methyl 2-benzimidazole carbamate, diethylstilbestrol and estradiol: Structural chromosomal aberrations, sister-chromatid exchanges, C-mitoses, polyploidies and micronuclei, Mutation Res., 156, 199–218.

Barrett, J.C., M. Oshimura, N. Tanaka and T. Tsutsui (1987) Genetic and epigenetic mechanisms of presumed nongenotoxic carcinogens, in: J.C. Barrett (Ed.), Mechanisms of Environmental Carcinogenesis, Vol. 1, CRC Press, Boca Raton, FL, pp. 1–15.

Bradley, M.O., V.I. Taylor, M.J. Armstrong and S.M. Galloway (1987) Relationship among cytotoxicity, lysosomal breakdown, chromosome aberrations and DNA double-strand breaks, Mutation Res., 189, 69–79.

Buenaventura, S.K., D. Jacobson-Kram, K.L. Dearfield and J.R. Williams (1984) Induction of sister chromatid exchange by diethylstilbestrol in metabolically competent hepatoma cell lines but not in fibroblasts, Cancer Res., 44, 3851–3855.

Carrano, A.V. and A.T. Natarajan (1988) Considerations for population monitoring using cytogenetic techniques, Mutation Res., 204, 379–406.

Chrisman, C.L. and A.P. Baumgartner (1979) Cytogenetic effects of diethylstilbestrol-diphosphate (DES-dp) on mouse bone marrow monitored by the micronucleus test, Mutation Res., 67, 157–160.

Drevon, C., C. Piccoli and R. Montesano (1981) Mutagenicity assays of estrogenic hormones in mammalian cells, Mutation Res., 89, 83–90.

Gonzalez, A., T.D. Oberley and J.J. Li (1989) Morphological and immunohistochemical studies of the estrogen-induced Syrian hamster renal tumor: Probable cell of origin, Cancer Res., 49, 1020–1028.

Henderson, B.E., R.K. Ross, M.C. Pike and J.T. Casagrande (1982) Endogenous hormones as a major factor in human cancer, Cancer Res., 42, 3232–3239.

Hill, A. and S. Wolff (1983) Sister chromatid exchanges and cell division delays induced by diethylstilbestrol, estradiol, and estriol in human lymphocytes, Cancer Res., 43, 4114–4118.

Hillbertz-Nilsson, K. and J.-G. Forsberg (1989) Genotoxic effects of estrogens in epithelial cells from the neonatal mouse uterine cervix: Modifications by metabolic modifiers, Teratogen. Carcinogen. Mutagen., 9, 97–110.

Husum, B., H.C. Wulf and E. Niebuhr (1982) Normal sister chromatid exchanges in oral contraceptive users, Mutation Res., 103, 161–164.

Kinsella, A.R. and M. Radman (1978) Tumor promoter induces sister chromatid exchanges: Relevance to mechanisms of carcinogenesis, Proc. Natl. Acad. Sci. USA, 75, 6149–6153.

Kirkman, H. (1959) Estrogen-induced tumors of the kidney in Syrian hamsters, J. Natl. Cancer Inst. Monogr., 1, 1–59.

Kochhar, T.S. (1985) Inducibility of chromosome aberrations by steroid hormones in cultured Chinese hamster ovary cells, Toxicol. Lett., 29, 201–206.

Kovacs, G. and S. Frisch (1989) Clonal chromosome abnormalities in tumor cells from patients with sporadic renal cell carcinomas, Cancer Res., 49, 651–659.

Li, J.J. and S.A. Li (1987) Estrogen carcinogenesis in Syrian hamster tissues: Role of metabolism, Fed. Proc., 46, 1858–1863.

Li, J.J. and S.A. Li (1994a) The effect of hormones on tumor induction, in: J.C. Arcos and M.D. Argus (Eds.), Chemical Induction of Cancer Modulation and Combination Effects, Elsevier, New York, in press.

Li, J.J. and S.A. Li (1994b) Estrogen carcinogenesis in the hamster kidney: A hormone-driven multi-step process, in: J. Huff, J. Boyd and J.C. Barrett (Eds.), Cellular and Molecular Mechanisms of Hormonal Carcinogenesis: Environmental Influences, Wiley-Liss, Philadelphia, PA, in press.

Li, J.J., A. Gonzalez, S. Banerjee, S.K. Banerjee and S.A. Li (1993) Estrogen carcinogenesis in the hamster kidney: Role of cytotoxicity and cell proliferation, Environ. Health Perspect., 101 (Suppl. 5), 259–264.

Li, S.A. and J.J. Li (1992) Metabolism of Moxestrol in the hamster kidney: Significance for estrogen carcinogenesis, in: J.J. Li, S. Nandi and S.A. Li (Eds.), Hormonal Carcinogenesis, Springer-Verlag, New York, pp. 110–117.

Llombart-Bosch, A. and A. Peydro (1975) Morphological, histochemical and ultrastructural observations of diethylstilbestrol induced kidney tumors in the Syrian golden hamster, Eur. J. Cancer, 11, 403–412.

Martin, C.N., A.C. McDermid and R.C. Gaines (1978) Testing of known carcinogens and noncarcinogens for their ability to induce unscheduled DNA synthesis in HeLa cells, Cancer Res., 38, 2621–2627.

Mehnert, K., G. Speit and W. Vogel (1985) Effect of diethylstilbestrol on the frequencies of sister chromatid exchange in vitro and in vivo, Cancer Res., 45, 3626–3630.

Oberley, T.D., T.D. Pugh, A. Gonzalez, S. Goldfarb, S.A. Li and J.J. Li (1989) Specific estrogen induced cell proliferation of cultured Syrian hamster proximal renal tubular cells in serum free chemically defined media, Proc. Nat. Acad. Sci. USA, 86, 2107–2111.

Oberley, T.D., A. Gonzalez, L.J. Lauchner, L.W. Oberley and J.J. Li (1991) Characterization of early kidney lesions in estrogen-induced tumors in Syrian hamster, Cancer Res., 51, 1922–1929.

Popescu, N.C. and J.A. Di Paolo (1988) Chromosome alterations in Syrian hamster cells transformed in vitro by sodium bisulfite, a nonclastogenic carcinogen, Cancer Res., 48, 7246–7251.

Racine, R.R. and B.P. Schmid (1983) DNA-damaging potential of diethylstilbestrol evaluated in the germ cell unscheduled DNA synthesis assay, Environ. Mutagen., 6, 211–218.

Rüdiger, H.W., F. Haenisch, M. Metzler, F. Oesch and H.R. Glatt (1979) Metabolites of diethylstilbestrol induce sister chromatid exchange in human cultured fibroblasts, Nature, 5730, 392–394.

Satya-Prakash, K.L., T.C. Hsu and W.J. Wheeler (1984) Metaphase arrest, anaphase recovery and aneuploidy induction in cultured Chinese hamster cells following exposure to mitotic arrestants, Anticancer Res., 4, 351–356.

Sawada, M. and M. Ishidate (1978) Colchicine-like effects of diethylstilbestrol on mammalian cells in vitro, Mutation Res., 57, 175–182.

Schimke, R.T., S.W. Sherwood, A.B. Hill and R.N. Johnston (1986) Overreplication and recombination of DNA in higher eukaryotes: Potential consequences and biological implications, Proc. Natl. Acad. Sci. USA, 83, 2157–2161.

Sharief, Y., A.M. Brown, L.C. Backer, J.A. Campbell, B. Westbrook-Colling, A.G. Kovacs and S. Frisch (1989) Clonal chromosome abnormalities in tumor cells from patients with sporadic renal cell carcinomas, Cancer Res., 49, 651–659.

Sutou, S.H. and Y. Arai (1975) Possible mechanisms of endoreduplication induction. Membrane fixation and/or disruption of the cytoskeleton, Exp. Cell Res., 92, 15–22.

Tsutsui, T., G.H. Degen, D. Schiffmann, A. Wong, H. Maizumi, J.A. McLachlan and J.C. Barrett (1984) Dependence on exogenous metabolic activation for induction of unscheduled DNA synthesis in Syrian hamster embryo cells by diethylstilbestrol and related compounds, Cancer Res., 44, 184–189.

Van Den Berghe, H. (1987) Chromosome abnormalities in solid tumors and their significance, Cancer Genet. Cytogenet., 28, 49–54.

Wolf, S. and B. Rodin (1978) Saccharin-induced sister chromatid exchanges in Chinese hamster and human cells, Science, 200, 543–545.

Worton, R.G. and C. Duff (1979) Karyotyping, in: W.B. Jakoby and I.H. Pastan (Eds.), Methods in Enzymology, Academic Press, New York, pp. 322–344.

Yang, A.H., J. Gould-Kostka and T.D. Oberley (1987) In vitro growth and differentiation of human kidney tubular cells on a basement membrane substrate, In Vitro Cell Dev. Biol., 23, 34–46.