

CHEMICALLY INDUCED CARCINOGENESIS: A COMPARISON OF 1-METHYL-1-NITROSOUREA, 7,12-DIMETHYLBENZANTHRACENE, DIETHYLNITROSO-AMINE AND AZOXYMETHANE MODELS (MINIREVIEW)

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The induction of mammary gland and other organ tumours by selected chemical carcinogens, 1-methyl-1-nitrosourea, 7,12-dimethylbenzanthracene, diethylnitrosoamine and azoxymethane is described and their application in experimental carcinogenesis research is discussed.

Key words: Chemically induced carcinogenesis – mammary gland – 1-methyl-1-nitrosourea, 7,12-dimethylbenzanthracene – diethylnitrosoamine – azoxymethane

Chemically induced carcinogenesis models in the rat are widely used for studying the biology of cancer and for developing and evaluating cancer prevention strategies. The most used chemical carcinogen models, 1-methyl-1-nitrosourea (MNU), 7,12-dimethylbenzanthracene (DMBA), diethylnitrosoamine (DEN) or azoxymethane (AOM) have been predominantly used in the investigation of a variety of novel cancer chemopreventive agents (Ip 1996).

1. 1-methyl-1-nitrosourea (MNU)

The carcinogenicity of MNU and N-ethyl-N-nitrosourea (ENU) was studied with the use of a hamster tracheal tumour model system. Hamsters received 15 or 10 once-weekly treatments with either 0.5 or 0.25 % solution of MNU and the experiment was terminated 9 months after the first intratracheal instillation. Other hamsters received 15 once-weekly treatments of a 0.5, 0.25, or 0.125% solution of ENU and were killed at 6 months. Treatment with MNU resulted in a dose-dependent induction of tracheal carcinomas; 94 % of the tumours induced were combined epidermoid and adenocarcinomas. Treat-

ment of hamsters with a 0.5, 0.25, 0.125 % solution of ENU induced an 83, 64, and 71 % incidence of benign tracheal tumours, respectively (papillomas and polyps). No tracheal carcinomas were induced by ENU (GRUBBS et al. 1981).

The efficacy of s.c. administration of MNU for the induction of mammary carcinomas was compared with the i.v. method of carcinogen injection in female Sprague-Dawley rats. Animals were injected at 50 days of age with 50 mg MNU per kg body weight. Animals were palpated for tumour detection weekly and the experiment was ended 180 days after injection with the carcinogen. Cancer incidences were similar, 95 versus 90 % in animals given MNU either s.c. or i.v. with an average of 3.9 and 3.9 cancers per rat, respectively. Time of tumour appearance was essentially identical under both treatment conditions. Using either method of carcinogen administration resulted in the induction of approximately 2.4 times more carcinomas in the cervical-thoracic mammary glands than in the abdominal-inguinal glands with no differences observed in cancer occurrence in the left versus the right mammary gland chains. The data indicate that the administration of

MNU is as effective and specific in the induction of mammary carcinomas (THOMPSON and MEEKER 1983).

In 1991, dose-response relationships for the induction of mammary tumours by a single i.p. injection of MNU were studied (THOMPSON and ADLAKHA 1991). Female Sprague-Dawley rats were given i.p. injections of 50, 37.5, 25, 12.5, or 0 mg MNU/kg body weight at 50 days of age. Animals were palpated for tumour detection twice weekly throughout a 28-week observation period. Administration of MNU i.p. caused no acute toxicity but induced both benign and malignant mammary tumours. However, malignant tumours appeared earlier and at a faster rate than benign tumours. The incidence and numbers of mammary carcinomas increased whereas median cancer-free time decreased with increasing dose of MNU. Approximately twice as many mammary cancers were observed in the cervical-thoracic as in the abdominal-inguinal mammary gland chains irrespective of carcinogen dose. The mammary tumour response attained via i.p. injection was similar but the coefficient of variation for tumour multiplicity within a carcinogen dose group was lower in comparison to that observed when MNU was administered i.v. or s.c. Among these techniques for carcinogen injection, the i.p. route is the most rapid method and offers an added advantage of ease of administration with quantitative, reproducible delivery of the desired amount of carcinogen and a decrease in variability of tumour response among animals within a treatment group (THOMPSON and ADLAKHA 1991).

Year later THOMPSON et al. (1992) made experiment to determine if the tumourigenic response to MNU in the mammary gland varied with age of administration and was dose dependent when the carcinogen was injected prior to 50 days of age. Using a recently developed method for mammary tumour induction, MNU was injected i.p. at doses ranging from 25 to 75 mg/kg at 35 days of age or 50 mg/kg at 28, 35 or 42 days of age. Treatment with MNU resulted in induction of both benign and malignant mammary tumours. The incidence of mammary gland adenocarcinomas was 100 % at and above the 50 mg/kg dose of MNU, irrespective of the age at which carcinogen was administered. The number of cancers increased in proportion to carcinogen dose, whereas cancer latency decreased as the MNU dose

increased. Metastases of mammary neoplasms to lung, liver and spleen were observed in rats injected with MNU at 35 or 42 days of age. These data indicate the dose responsiveness of MNU-induced mammary carcinogenesis in rats initiated prior to 50 days of age, the lack of effect of age at initiation if prior to 50 days on final tumour outcome, and that the age at which MNU is injected may affect the metastatic potential of the mammary carcinomas that are induced (THOMPSON et al. 1992).

The testing the effects of MNU are usually performed on 50 day old Sprague-Dawley rats. However, in some experiments 120 day old rats were used to better compare with the incidence of breast carcinoma in human. The administration of one i.v. dose of 50 mg MNU/kg to 120 day old rats Sprague-Dawley induced 75-95 % incidence of tumours 180 days post-carcinogen treatment. MNU-induced carcinomas, in contrast to DMBA-induced carcinomas, are more aggressive, adenocarcinomas and hormone-dependent (MOON et al. 1992).

In order to obtain an experimental model RIVERA et al. (1994) induced mammary tumours in female Sprague-Dawley rats. The carcinogen MNU was injected intraperitoneally at doses of 50 mg/kg body weight when animals were 50, 80 and 110 day old. Tumour sizes were measured with a caliper and their growth parameters and histopathological properties were tested. For 100 rats, 88.4 % of developed lesions were ductal carcinomas, histologically classified as 52.8 % cribriform variety, 30.6 % solid carcinoma. Metastases in liver, spleen and lung were present. Other primary tumours were detected with low incidence (RIVERA et al. 1994).

The effectiveness of the direct application of crystalline MNU onto the mammary gland was compared with the systemic intraperitoneal administration method for the induction of mammary carcinomas in female Sprague-Dawley rats. The 10 mg crystalline MNU was dusted directly onto the right-inguinal mammary gland, or 50 mg/kg body weight MNU solution was given i.p. at 50 days of age. Animals were palpated for tumour detection twice weekly and killed when the tumour reached 1-2 cm in diameter or were necropsied 30 weeks after carcinogen treatment. The cumulative incidence of mammary carcinoma was high in the dusting and the i.p. groups (12/12; 100% and 11/13; 84 %, respectively). all of the

78 tumours produced by dusting were adenocarcinomas. By contrast, 40 tumours produced i.p. were adenocarcinomas, 1 was fibroadenoma, and 5 were lactating adenomas. The dusting groups showed a high number of carcinoma per rats (6.5 vs. 3.6) and short cancer latency than the i.p. groups. In both groups, adenocarcinomas displayed various degrees of differentiation but no evidence was found for metastasis. For MNU-administration, the direct dusting technique is an effective method and offers added advantages of ease for the induction of mammary carcinomas in rats (TAKAHASHI et al. 1995).

THOMPSON et al. (1995) reported a carcinogen induction protocol that defines conditions under which approximately 38 % of detectable lesions in the abdominal-inguinal mammary glands were histologically classified as either intraductal proliferations or ductal carcinoma *in situ*. The remainder of the lesions were classified as carcinomas. This response was observed in a group of 30 female Sprague-Dawley rats injected i.p. with 50 mg MNU/kg body wt at 21 days of age. The experiment was terminated 35 days following carcinogen administration. The first palpable tumour, histologically classified as an adenocarcinoma, was observed 30 days post carcinogen administration. When the experiment was terminated (35 days post MNU), the cumulative incidence of palpable carcinomas was 60 %. The MNU injection resulted in >99 % incidence of palpable mammary gland tumours that were malignant (THOMPSON et al. 1995).

In 1998, THOMPSON et al. (1998a) designed an experiment to examine the question of whether a mammary epithelial cell's independence from hormonal requirements is established at the time of carcinogenic initiation, or whether the emergence of hormone independence is associated with the process of tumour progression. Two experiments were conducted in Sprague-Dawley rats injected with 50 mg MNU/kg body wt at 21 days of age. In the first experiment 47 animals were ovariectomized after the detection of a mammary tumour of palpable size. Forty-six of the 47 tumours assessed, all of which were subsequently classified as mammary gland adenocarcinomas, regressed to <50 % of their initial volume within 14 days of bilateral ovariectomy. In 2nd experiment a total of 60 rats were ovariectomized 7 days after MNU was injected. At 35 days post car-

cinogen ovariectomized animals had a higher incidence of intraductal proliferations than sham-operated controls ($P=0.03$); there was no effect of ovariectomy on the incidence of ductal carcinoma *in situ* or carcinoma. The multiplicity of intraductal proliferations was increased by 58 % in ovariectomized rats ($P=0.12$), but the number of mammary carcinoma per rat was reduced (3.8 vs. 1.57; $P=0.02$). These data are consistent with the hypotheses that the progression of pre-malignant to malignant lesions is inhibited in the mammary gland by ovariectomy and that the hormone independent phenotype can be conferred at the time of carcinogenic initiation (THOMPSON et al. 1998a).

In 1998, experiment was designed to determine the latency period between carcinogen administration and the occurrence of each of these types of lesion. A total of 150 female Sprague-Dawley rats were injected i.p. with 50 mg 1-methyl-1-nitrosourea (MNU)/kg body wt at 21 days of age. Groups of 30 rats each were killed at 7, 14, 21, 28 and 35 days post-carcinogen. Mammary intraductal proliferations were the first detected lesions and were observed in 20 % of the animals at 14 days following carcinogen administration. At 21 days post-carcinogen ductal carcinomas *in situ* and adenocarcinomas were observed. The number of each type of lesion increased with time post-carcinogen, but the temporal pattern of occurrence was different among lesion types. Ductal carcinomas *in situ* represent one pathway of morphological progression by which intraductal proliferations evolve into invasive carcinomas, but that this lesion type, as currently defined histologically, may not be an obligatory intermediate in morphologic progression (THOMPSON et al. 1998b).

1.1. The molecular basis of the MNU-induced carcinogenesis

The treatment of MNU transforms mouse mammary epithelial cells to preneoplastic and neoplastic states. Mammary carcinomas arising from MNU-induced hyperplastic alveolar nodules contain transformed c-Ki-ras proto-oncogene with the presence of a specific G-35 → A-35 point mutation in codon 12, which results in the substitution of the normal glycine with an aspartic acid. The specific c-Ki-ras mutation is

a preneoplastic event in MNU-induced mouse mammary carcinogenesis (MIYAMOTO et al. 1990).

During tumour progression in rat the preferential amplification of the mutated Ha-ras allele (GGA → GAA; 12-th codon), cyclin D1 gene (PRAD-1), insulin-like growth factor gene (IGF2), loss of expression of the mitogenic growth factor gene, heparin-binding growth factor midkine gene (MK) and mutation in the tumour suppressor gene, p53, are seen in the mammary tumours (SUKUMAR et al. 1995; CHEN et al. 1996).

In experimental model of mammary carcinogenesis in female Sprague-Dawley rats induced by two intrajugular injection of MNU (50 mg/kg), 7 days apart, beginning at 44-49 days of age, the accumulation of p53 protein in cells was demonstrated in 22 from 37 rat mammary tumours. These results indicate that elevated cellular content of p53 is a common event in invasive palpable mammary tumours induced by MNU in this model system (CRIST et al. 1996).

In 1997 LU et al. (1997) demonstrated overexpression of the cDNA fragments of gene transcripts: rat homologues of human galectin-7 gene, the human/mouse melanoma inhibitory activity/bovine chondrocyte-derived retinoic acid sensitive protein gene, the mouse stearyl-CoA desaturase-2 gene, and the mouse endo B cytokeratin/human cytokeratin-18 gene in MNU-induced rat mammary carcinogenesis model. These genes may represent mammary carcinoma-specific molecular markers that may be helpful in investigations of mammary carcinogenesis and its prevention (LU et al. 1997).

In 1997 WIDSCHWENDTER et al. (1997) describe the completely loss of RAR- β expression in breast tumours of 13 from 14 patients. One possibility to explain the suppression of RAR- β is a mutation in the promoter region on chromosome 3p24 (WIDSCHWENDTER et al. 1997).

2. 7,12-dimethylbenzanthracene (DMBA)

There is good evidence in some species, including rats that circannual rhythms are innate and can occur even under constant environmental conditions. Such circannual rhythms, e.g. in hormone levels and immune system function, may influence tumourigenesis. This prompted LOSCHER et al. (1997) to study

7,12-dimethylbenzanthracene (DMBA)-induced mammary carcinogenesis at different seasons of the year in female Sprague-Dawley rats under constant environmental conditions (photoperiod, temperature, air humidity, food). DMBA was administered orally at a dose of 5 mg per rat at the first day of the experiment and then at weekly intervals up to a total dose of 20 mg per rat. Rats were palpated once weekly for the presence of mammary tumours. When the experiment was performed twice within 2 years during the same season (spring/summer), tumour incidence (56 and 61%) and tumour burden were almost equal. However, the same experiment performed in autumn yielded a significantly lower tumour incidence (34%) and tumour burden. The data indicate a seasonal variation in the development and growth of DMBA-induced breast cancer in Sprague-Dawley rats. One possible explanation for this phenomenon may be the seasonal variation in pineal melatonin production and immune function previously reported in rodents under constant environmental conditions (LOSCHER et al. 1997).

The combined application of MNU and DMBA was compared with the administration of each carcinogen alone as to the effectiveness of the induction of mammary carcinomas in female Sprague-Dawley rats. At 50 days of age, group 1 received 30 mg/kg MNU intraperitoneally (i.p.), group 2 received 30 mg/kg DMBA i.p., group 3 received 60 mg/kg MNU i.p., group 4 received 60 mg/kg DMBA i.p., group 5 received 30 mg/kg MNU followed by 30 mg/kg DMBA i.p., group 6 received 30 mg/kg MNU i.p. and then 30 mg/kg DMBA intravenously (i.v.) and group 7 remained untreated. Animals were killed when the largest mammary tumour reached 1-2 cm in diameter or were necropsied when they were 30 weeks of age. MNU i.p. produced no deaths (groups 1 and 3), however, the i.p. administration of DMBA induced death due to peritonitis (groups 2, 4 and 5), whereas the i.v. administration of DMBA suppressed the death (group 6). All of the tumours produced by MNU were adenocarcinomas of mammary origin. In contrast, DMBA produced tumours of other than mammary origin. The combined treatment with DMBA and MNU increased the mammary carcinogenic effect; it significantly increased the mean number of mammary cancers per rat (SHIRAI 1997).

3. Diethylnitrosoamine (DEN)

DEN is widely used chemical carcinogen in models of carcinogenesis of liver and esophagus.

Basophilic hepatic foci, nodules, and trabecular hepatocellular carcinomas, collectively referred to as focal hepatic lesions, were induced by single injections of 5.0 mg DEN/g body weight in 15-day-old B6C3 F1 mice. The only observable acute hepatic toxic effect of DEN, a mild steatosis, was noted at 3 days, but this had disappeared by 7 days following injection. Basophilic foci, composed entirely of altered hepatocytes characterized by their abundant cytoplasmic RNA, a high nuclear to cytoplasmic ratio (two times greater than normal), were first noted at 10 weeks. During the course of the study, basophilic foci appeared to increase in size and number. Invasion of hepatic veins by basophilic foci, first noted at 10 weeks, was prominent by 20 weeks and indicated that many of the lesions manifested this characteristic of malignancy well in advance of the anaplastic features that are also diagnostic of hepatocellular carcinoma (GOLDFARB et al. 1983).

In 1986 DRIVER et al. (1986) have investigated using an experimental model of rat hepatocarcinogenesis with one i.p. dose of DEN, that low doses of DEN resulted in the formation of hyperplastic nodules, but only the high dose of DEN (30 mg/kg) resulted in hepatocarcinoma formation (DRIVER et al. 1986).

Diethylnitrosamine (DEN) when given in drinking water at the dose of 4 mg/kg body weight/day, induced esophageal papillomas consistently in 100% of the ICRC mice, an inbred strain that exhibits megaesophagus with markedly hyperplastic mucosa. Tumours were produced, even at a very low cumulative dose of 28 mg/kg body weight. DEN-induced carcinogenesis in ICRC mouse thus provides a much needed animal model to study esophageal tumorigenesis, including the two-stage carcinogenesis (GHAISAS et al. 1989).

4. Azoxymethane (AOM)

Azoxymethane-induced carcinogenesis is very useful model of colon-rectal tumorigenesis in rats.

Male Fischer rats, 6 weeks old, were injected once with one of five doses of azoxymethane (5-25 mg/

kg). There was a dose response to the carcinogen as determined by weight gain and tumour induction. Rats given the three highest doses developed tumours of the gastrointestinal tract, auditory sebaceous glands, kidney, liver, and preputial gland, whereas rats receiving the lowest doses had tumours mainly of the intestine. Chronic liver lesions in high-dose rats were cirrhosis with megalocytosis, mild fibrosis, nodular hepatocellular hyperplasia, and hyperplasia of bile ductules (WARD 1975).

The histogenesis of large intestinal carcinomas was studied by use of a low dose (2 mg/kg/wk) of AOM administered to inbred F344 rats. In marked contrast to the standard dose (8 mg/kg/wk) studies, low-dose AOM induced very high incidence of readily metastasizing mucinous adenocarcinomas that were extremely aggressive. The carcinomas, microscopic and macroscopic, were localized predominantly in the proximal large intestine (SHAMSUDDIN and HOGAN 1984).

In 1985, ten-week-old male F344 rats were given 10 weekly s.c. injections of AOM at doses of 3, 7, or 14 mg/kg body weight and were killed 1 week or 15 weeks after the last AOM dose. One week post-carcinogen treatment, dose-dependent features of colonic epithelial cells were identified: mitochondrial injury, nuclear pleomorphism and loss of polarity. Because these dose-dependent features were present only shortly after AOM administration, they appeared to be manifestations of carcinogen toxicity and associated reactive, regenerative epithelial changes. By contrast, 15 weeks after last dose of AOM were identified: dose-dependent increased numbers of epithelial cells with enlarged nucleoli, reduced numbers of goblet cells, and reduced numbers of apical cytoplasmic vacuoles. Because these features persisted in a dose-dependent manner for many weeks after the last dose of AOM and were present after the latent period to tumor formation, they appear to be morphologic precursors to colonic carcinogenesis in the model (PAN et al. 1985).

The liver is the most frequent site of metastases in colon cancer (NORDLINGER et al. 1991). Any suitable animal model has been not available until 1995 to help improve the treatment of liver metastases or their prevention after resection of a primary colon cancer. Therefore, NORDLINGER et al. (1991) have designed an experiment to develop a model of colon cancer

induced by AOM in the rat and to study the outcome after surgical resection alone or in association with intraperitoneal chemotherapy by 5-fluorouracil (5-FU). Male Wistar rats received subcutaneous AOM (10 mg/kg body weight/week) for 12 weeks. Rats with isolated colon cancer underwent total colectomy; then rats with no metastases were randomized into two groups: surgery alone or surgery plus 5-FU (5 mg/kg body weight/day) for 5 days after surgery. Peritoneal carcinomatosis and liver metastases were more frequent in the control group than after adjuvant treatment with 5-FU (27.7 % versus 0 %). The rates of peritoneal and hepatic recurrence after resection of the primary cancer indicate that the model mimics the natural history of human colon cancer (NORDLINGER et al. 1991).

In our recent experiments, female Sprague-Dawley rats were injected intraperitoneally with MNU (50 mg.kg⁻¹ body weight) at the 33, 40, 47, 54 and 61 day of age in the first experiment of experiments and at 50th and 113th day in the second experiment. On the 117th day (first experiment) and on the 153rd day of age in the second experiment the rats were sacrificed by decapitation and their mammary glands were evaluated both macroscopically and microscopically for the presence of grossly detectable mammary tumours. We have found that the final incidence of palpable carcinomas was ranging from 60 % to 76 %. All microscopically evaluated tumours were malignant. In both experiments, various combinations of papillary, cribriform or comedo patterns of ductal carcinomas occurred in tumours. Among the total number of lesions classified the percentage of invasive tumours were ranged from 35 % to 47 %, and no metastasis was observed in other organs in MNU treated animals (LISKA et al. 2000).

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References

- CHEN Y, MCKENZIE KE, ALDAZ CM, SUKUMAR S: Midkine in the progression of rat N-nitroso-N-methylurea-induced mammary tumors. *Mol Carcinog* **17**, 112-6, 1996
- CRIST KA, FULLER RD, CHAUDHURI B, CHAUDHURI P, YOU M: Brief communication, P53 accumulation in N-methyl-N-nitrosourea-induced rat mammary tumors. *Toxicol Pathol* **24**, 370-5, 1996
- DRIVER HE, MCLEAN AE: Dose-response relationships for initiation of rat liver tumours by diethylnitrosamine and promotion by phenobarbitone or alcohol. *Food Chem Toxicol* **24**, 241-5, 1986
- GHAISAS S, SARANATH D, DEO MG: ICRC mouse with congenital mega-esophagus as a model to study esophageal tumorigenesis. *Carcinogenesis* **10**, 1847-54, 1989
- GOLDFARB S, PUGH TD, KOEN H, HE YZ: Preneoplastic and neoplastic progression during hepatocarcinogenesis in mice injected with diethylnitrosamine in infancy. *Environ Health Perspect* **50**, 149-61, 1983
- GRUBBS CJ, BECCI PJ, THOMPSON HJ, MOON RC: Carcinogenicity of N-methyl-N-nitrosourea and N-ethyl-N-nitrosourea when applied to a localized area of the hamster trachea. *J Natl Cancer Inst* **66**, 961-5, 1981
- IP C: Mammary tumorigenesis and chemoprevention studies in carcinogen-treated rats. *J Mammary Gland Biol Neoplasia* **1**, 37-47, 1996
- LISKA J, GALBAY S, MACEJOVA D, ZLATOS J, BRITKO J: Histopathology of mammary tumours in female rats treated with 1-methyl-1-nitrosourea. *Endocr Regul* **34**, 91-96, 2000
- LOSCHER W, MEVISSSEN M, HAUSSLER B: Seasonal influence on 7,12-dimethylbenz[a]anthracene-induced mammary carcinogenesis in Sprague-Dawley rats under controlled laboratory conditions. *Pharmacol Toxicol* **81**, 265-70, 1997
- LU J, PEI H, KAECK M, THOMPSON HJ: Gene expression changes associated with chemically induced rat mammary carcinogenesis. *Mol Carcinog* **20**, 204-15, 1997
- MIYAMOTO S, SUKUMAR S, GUZMAN RC, OSBORN RC, NANDI S: Transforming c-Ki-ras mutation is a preneoplastic event in mouse mammary carcinogenesis induced in vitro by N-methyl-N-nitrosourea. *Mol Cell Biol* **10**, 1593-9, 1990
- MOON RC, KELLOFF GJ, DETRISAC CJ, STEELE VE, THOMAS CF, SIGMAN CC: Chemoprevention of MNU-induced mammary tumors in the mature rat by 4-HPR and tamoxifen. *Anticancer Res* **12**, 1147-53, 1992
- NORDLINGER B, PANIS Y, PUTS JP, HERVE JP, DELELO R, BALLEF F: Experimental model of colon cancer: recurrences after surgery alone or associated with intraperitoneal 5-fluorouracil chemotherapy. *Dis Colon Rectum* **34**, 658-63, 1991

- PAN Q, HAMILTON SR, HYLAND J, BOITNOTT JK: Effects of carcinogen dosage on experimental colonic carcinogenesis by azoxymethane: an ultrastructural study of grossly normal colonic mucosa. *J Natl Cancer Inst* **74**, 689-98, 1985
- RIVERA ES, ANDRADE N, MARTIN G, MELITO G, CRICCO G, MOHAMAD N, DAVIO C, CARO R, BERGOC RM: Induction of mammary tumors in rat by intraperitoneal injection of NMU: histopathology and estral cycle influence. *Cancer Lett* **86**, 223-8, 1994
- SHAMSUDDIN AM, HOGAN ML: Large intestinal carcinogenesis. II. Histogenesis and unusual features of low-dose azoxymethane-induced carcinomas in F344 rats. *J Natl Cancer Inst* **73**, 1297-305, 1984
- SHIRAI K: Synergistic effect of MNU and DMBA in mammary carcinogenesis and H-ras activation in female Sprague-Dawley rats. *Cancer Lett* **120**, 87-93, 1997
- SUKUMAR S, MCKENZIE K, CHEN Y: Animal models for breast cancer. *Mutat Res* **333**, 37-44, 1995
- TAKAHASHI H, UEMURA Y, NAKAO I, TSUBURA A: Induction of mammary carcinomas by the direct application of crystalline N-methyl-N-nitrosourea onto rat mammary gland. *Cancer Lett* **92**, 105-11, 1995
- THOMPSON HJ, MEEKER LD: Induction of mammary gland carcinomas by the subcutaneous injection of 1-methyl-1-nitrosourea. *Cancer Res* **43**, 1628-9, 1983
- THOMPSON HJ, ADLAKHA H: Dose-responsive induction of mammary gland carcinomas by the intraperitoneal injection of 1-methyl-1-nitrosourea. *Cancer Res* **51**, 3411-5, 1991
- THOMPSON HJ, ADLAKHA H., SINGH M: Effect of carcinogen dose and age at administration on induction of mammary carcinogenesis by 1-methyl-1-nitrosourea. *Carcinogenesis* **13**, 1535-9, 1992
- THOMPSON HJ, MCGINLEY JN, ROTHHAMMER K, SINGH M: Rapid induction of mammary intraductal proliferations, ductal carcinoma in situ and carcinomas by the injection of sexually immature female rats with 1-methyl-1-nitrosourea. *Carcinogenesis* **16**, 2407-11, 1995
- THOMPSON HJ, MCGINLEY JN, ROTHHAMMER K, SINGH M: Ovarian hormone dependence of pre-malignant and malignant mammary gland lesions induced in pre-pubertal rats by 1-methyl-1-nitrosourea. *Carcinogenesis* **19**, 383-6, 1998a
- THOMPSON HJ, MCGINLEY JN, WOLFE P, SINGH M, STEELE VE, KELLOFF GJ: Temporal sequence of mammary intraductal proliferations, ductal carcinomas in situ and adenocarcinomas induced by 1-methyl-1-nitrosourea in rats. *Carcinogenesis* **19**, 2181-5, 1998b
- WARD JM: Dose response to a single injection of azoxymethane in rats. Induction of tumors in the gastrointestinal tract, auditory sebaceous glands, kidney, liver and preputial gland. *Vet Pathol* **12**, 165-77, 1975
- WIDSCHWENDTER M, BERGER J, DAXENBICHLER G, MULLER-HOLZNER E, WIDSCHWENDTER A, MAYR A, MARTH C, ZEIMET AG: Loss of retinoic acid receptor beta expression in breast cancer and morphologically normal adjacent tissue but not in the normal breast tissue distant from the cancer. *Cancer Res* **57**, 4158-61, 1997

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