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Review paper

The effects of sex steroids on colon carcinogenesis

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Numerous reports on colon carcinogenesis reveal gender differences in the incidence and location of tumors. A large number of the presented studies suggest that sex-steroids have a considerable effect on tumorigenesis of the large bowel. To clarify the, so far, not fully understood mechanisms and somewhat conflicting information about the possible hormone action, it is important to discuss two points: first, the reliability of the currently used experimental animal models; and second, what is known concerning the role of sex steroids in colon carcinogenesis.

Key words: Sex steroids, colon carcinogenesis, experimental animal model.

Introduction

Human colorectal cancer has long been thought of as being mainly caused by environmental factors.[1] Experimental and clinical findings suggest that human breast cancer and colonic cancer might share common etiologic factors.[2,3] Recently, reproductive factors have been postulated to play an important role in the etiology of colonic cancer, similar to breast cancer.[4] These associations raised the question of the role of gender steroid hormones in colonic carcinogenesis. The presence of specific receptor proteins found in at least one-third of colonic tumors lent further support to this theory.[5,6]

Studies of the different human diseases require reliable animal models. The ideal animal model should be able to mimic human tumors in biological behavior, morphology, pathophysiology and biochemical changes. Before 1965 there was no satisfactory animal model for colorectal cancer and therefore there was little experimental research on this subject. The development of animal models for studying the etiology of colon cancer started in 1962 when cycasin, a natural component of the cycad nut, was found to have a carcinogenic effect in rodents (Figure 1).[7] Gradually, more efficient and more potent colonic carcinogens were developed, mainly synthetic derivatives of cycasin (Figure 1).[8,9] Together with two further substances from different chemical origins, methyl-nitroso-urea (MNU) and methyl-nitro-nitroso-guanidine (MNNG), the three cycasin derivatives are currently the most frequently used in studies of experimental colon carcinogenesis.[8,10] The induced tumors resemble human colorectal cancer macroscopically. Microscopically the benign tumors show the same range of differentiation as in man—tubular, villous, tubulo-villous. The malignant neoplasms can be classified by the same criteria as those applied to colonic cancer in man (infiltration and penetration of the muscular mucosa).[10]

Clinical trials revealed sex-related differences in the incidence of colorectal cancer. Women tend to have a higher incidence for cancer in the right colon, in contrast to men, who mainly develop cancer in the left colon and rectum.[11] Age-dependent sex differences were also found.[4] Men under 35 years of age show a higher rate of colorectal cancer, whereas in the range 35–55 years the situation turns vice versa (females > males). Above 55 years of age colorectal cancer is predominantly found in men.

Epidemiological studies show higher incidence rates of (large) bowel cancer in premenopausal women than in men of the same age. Moreover, lower incidences of these tumors have been reported in women of higher parity, and nulliparity

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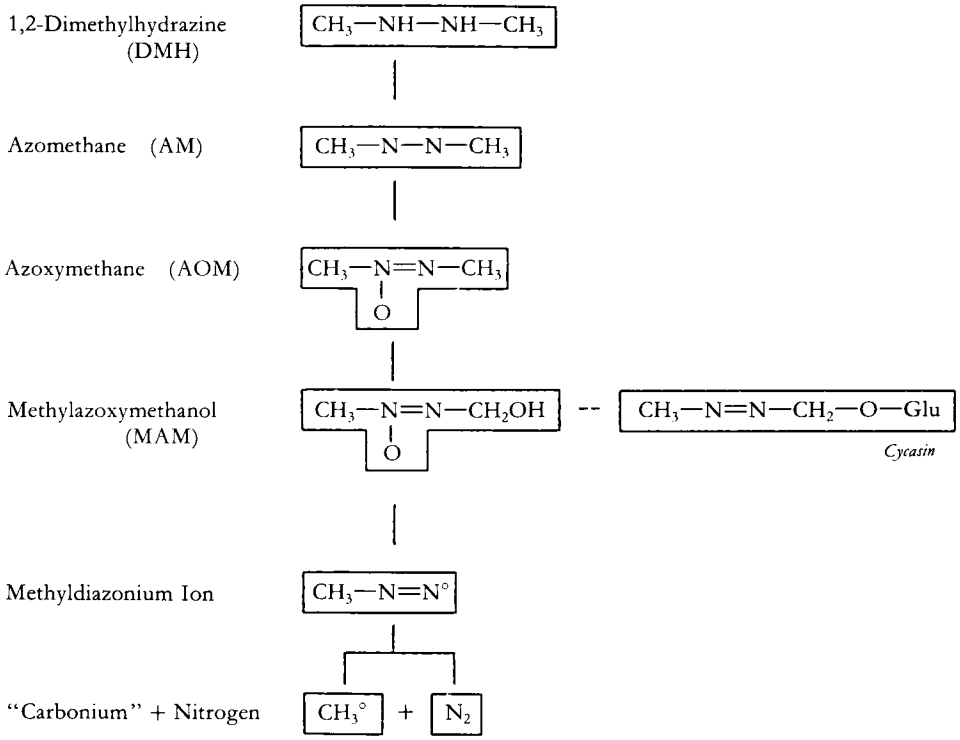

Figure 1. Cycasin and the metabolic pathway of its synthetic derivatives.

in particular seems to represent a risk factor for developing colonic cancer.[11] These findings have been explained as due to a variety of non-epithelial sex characteristics such as differences in bile metabolism, colonic bacteria and fecal transmit times.

Chemical carcinogenesis in rodents revealed sex differences in tumor incidences.[12,13] In experimental colon carcinogenesis, incidence rates of chemically induced colonic carcinomas are diminished by gonadectomy and are increased by hormone substitution.[13,14] Furthermore, in experimental animals, androgens as well as estrogens have shown influences on carcinogenesis and many studies have now revealed (sex) steroid hormone receptors in colorectal tumor cells. Thus, an effect, not yet fully understood, of these hormones on the epithelium in the process of malignant transformation must be considered.

Colon carcinogens

Reliable tumor induction in experimental colon carcinogenesis is provided by derivatives of cycasin, such as DMH and AOM (Figure 1), and alkylnitrosamines, such as MNU and MNNG. These substances represent two types of chemicals for tumor induction—one requiring metabolic activation (DMH and AOM) and one directly active (MNU and MNNG).

Mode of action

Chemical carcinogens produce tumors by modifying the genome of the cell, resulting in eventual alterations of the cell phenotype, such as loss of differentiation, invasiveness, or abnormal proliferative behavior.[1] The carcinogenic action of hydrazine derivatives appears to involve methylation of colonic epithelial cell DNA that ultimately results in changes of epithelial proliferation.[15]

Animals

Animals mainly used in studies of experimental colon carcinogenesis are rodents: rats (e.g. Fischer 344, Sprague–Dawley); mice (e.g. Balb/c); hamsters; and, to a much lesser extent, marmosets.[16] Rodents provide specific advantages in comparison to other animal species because:

- Rarely is there a spontaneous development of colorectal tumors in rodents;[16]

- Susceptibility to the carcinogens used (DMH, AOM, etc.) in rodents is high;[10,17]
- Even when failing to reproduce the human adenoma–carcinoma sequence, chemically-induced tumors of the large bowel in rodents are similar in histology and gross macroscopic properties to their human counterparts;[10,18]
- Housing and feeding of rodents is not a major problem, even when large numbers of animals are used.

Steroid hormones

It has been reported that sex hormones appear to affect colon carcinogenesis.[13,19] Experimental studies with animals initiated to clarify the obvious preponderance of right-sided colon cancer in women and left-sided colon and rectum carcinomas in men led to the detection of specific steroid hormone receptors in the human intestine and in human colon tumors.[5,10,20–23] Since the detection of these hormone receptors in both human colorectal tissue and tissue from human colorectal adenocarcinomas, androgens and estrogens have been suggested to exert an important role in colonic carcinogenesis.[24] Subsequently, experimental colon carcinogenesis studies in animals were undertaken which revealed the existence of specific androgen receptor proteins in chemically-induced colonic carcinomas.[25,26] Consequently, proliferative responses of the normal and abnormal intestinal crypt epithelium to altered steroid levels in combination with surgical manipulations to gender steroid-producing glands and/or administration of antagonists to those steroids were examined in several studies.[27,28] They revealed epithelial changes mostly in jejunal crypts with only few, or no, changes in the large bowel tract.

In experimental colon carcinoma studies, hormonal manipulations have been reported to influence tumor yield. Castration was thought to cause a reduction in tumor incidence;[14] however, the results presented are still controversial. Some authors were able to demonstrate the protective effects of androgen treatment against experimental colon carcinogenesis,[13,14] while other studies showed a promoting role of androgens on colorectal tumors.[19,25] Some findings suggest different influences of androgens on the site distribution in colonic tumorigenesis. It was found that hormone substitution in castrated rats significantly influences the site distribution of colonic neoplasms. Other hormonal manipulations did not have any significant effect in this respect.[29]

The receptor proteins were also the subject of experimental studies. The increase in carcinogen-induced receptors in animals could be demonstrated.[25,30] In addition, hormonal manipulations like gonadectomy resulted in the detection of increased amounts of androgen receptors in normal colonic tissue.[25] Gonadectomized animals which received additional carcinogen treatment showed a much higher rise in receptor density than in normal colon tissue. The highest amounts of androgen receptor proteins were found in chemically-induced colonic tumors;[25,26,28] however, conflicting results were also presented.[19] In this respect it is interesting to note that in studies by Metha *et al.*[25] and Odagiri *et al.*[30] the receptor level in tumors was said to be higher than in adjacent parts of the colonic mucosa, whereas Krelenbaum *et al.*[26] found similar levels in both tissues. Izbicki *et al.*[19] reported that the receptor level was higher in the adjacent mucosal tissue than in the tumor. However, both Odagiri and Izbicki found that carcinogen-treatment was associated with an increase in mucosal androgen receptors in comparison to non-treated controls.

Estrogen-receptor positive tumors, like carcinomas of the breast and uterus, are known to be highly sensitive to treatment with anti-estrogens.[31] Patients suffering from breast cancer have been shown to bear an increased risk of developing colorectal cancer.[32] Since estrogen receptors were also found in gastrointestinal tissues and tumors[5,33] the existence of genetic links between these two types of cancer is assumed, supporting considerations of a possible common etiologic origin.[2,3] Consequently anti-estrogens were also thought to have a possible therapeutic effect on colorectal cancer. The anti-estrogen *tamoxifen*, established in the therapy of breast cancer, was used in clinical trials with metastatic colorectal cancer. A new anti-estrogen, *droloxifen*, is presently undergoing a clinical trial in our department. Early results will shortly be presented.

Experimental animal studies to investigate effects of droloxifen on transplanted human colorectal tumors on nude mice have just been initiated in our laboratories. Additional investigations are still under way, for it is not yet clearly understood what role steroid hormones may finally play and what mechanisms they might induce in colonic carcinogenesis.

Sex-steroid receptors have also been identified in cells of the liver, pancreas and stomach of humans and animals.[34–38] Consequently colon tissue is not the only target for investigations of sex-steroid

effects. Mainly, the liver and pancreas are the bases for studies in numerous research projects, as can be seen by a review of the literature over recent years. Prostate and related neoplasms have been studied to a much lesser extent.[39,40] However, conflicting results about the role of sex steroids in colon-carcinogenesis are well presented for these organs.

Concerning liver tumorigenesis, the maintenance of high liver estrogen levels is thought to exert a protective effect against the development of hepatocellular carcinoma.[41] In contrast to these results, Wilkinson *et al.*[42] postulate a more synergistic effect of estrogens in hepato-carcinogenesis and a dependence of those tumors on androgens. A Japanese study group reported results showing decreasing estrogen receptor levels in hepatic carcinoma, thus assuming an ineffectiveness of anti-estrogen therapy for this type of cancer.[35]

Substantial evidence is said to exist concerning hormonal influences on pancreatic carcinogenesis.[43] However, research on the effects of sex steroids on pancreatic tumorigenesis seems to be at a preliminary stage and the results so far do not sound very encouraging. Several experimental studies[44–47] support the existence of steroid hormone receptors in hamster and rat pancreas. Some of these studies revealed gender-related differences in sex-steroid effects on pancreatic cancer.[44,46,47] With the exception of Pousette and co-workers[48] who think of elevated estrogen binding proteins (EBP) as a possible marker of cellular damage in the pancreas, no publication dealing with possible therapy or therapeutic strategies for pancreatic cancer is to be found in the reviewed literature.

Very few studies concerning prostate cancer have been published in recent years. They mostly deal with the androgenic situation in man suffering from this disease. Some of the results published indicate that men with prostatic cancer have elevated testosterone levels without any alteration in estrogen,[40] whereas another study group assumes following their data that the combination of hormonal stimulation and chemotherapy has a potent antitumorous effect on advanced prostate cancer refractory to orchiectomy.[39]

Other mechanisms

Further mechanisms of inhibition of chemically induced carcinogenesis are possible:

1. Antioxidants might prevent metabolic oxidation of carcinogens, thus preventing them from reaching their ultimate active form. In experimental animal studies, supplementation of selenium (an antioxidant) reduced chemically-induced colon tumor incidence from 87% in DMH-only treated animals to 40% in DMH-plus selenium treated rats.[49] Blocking of oxidation-steps in the metabolic pathway of the carcinogen is assumed; however, the exact mechanism has not yet been elucidated.

Vitamin E, also an antioxidant, appears to act in a similar manner to selenium.[50]

Vitamin C is presumed to act by directly interfering with the carcinogen, thus blocking the formation of the ultimately active carcinogenic compound.[49] In a clinical trial, vitamin C was reported to reduce the number of polyps in familial polyposis patients with multiple polyps who had undergone ileorectal anastomosis.[21,51]

Disulfiram ('Antabuse') inhibits DMH- and, to a lesser extent, AOM-induced experimental carcinogenesis by blocking certain steps of oxidation.[42] Exact knowledge of the mechanism is still lacking. Whether this agent could have a possible therapeutic basis in the treatment of human colorectal cancer still needs further investigation.

2. Compounds that influence microsomal enzymatic activity (yielding the same result as that shown in point 1).

3. Agents that prevent alterations in membrane structure and function, thus protecting the colonic mucosal wall from being infiltrated by possible carcinogenic substances.

4. Compounds that prevent active metabolites of carcinogens from binding at critical target sites (e.g. nucleic acids).

5. An increased level of prostaglandins observed in patients with colorectal carcinomas gave the rationale for investigations of their role in carcinogenesis.[53] The use of piroxicam and indomethacin respectively was based on the concept that these drugs may exert an inhibitory effect on endogenous prostaglandin synthesis, thus suppressing its possible promoting role in tumorigenesis.[54,55]

It could be demonstrated that increasing dietary doses of piroxicam lowered the colon tumor incidence in a dose-dependent manner.[53] The exact mechanism by which piroxicam inhibits chemically-induced colon carcinogenesis, however, has not yet been clarified.

Indomethacin inhibits intestinal tumor induction by several carcinogens, but tumors occur immediately when the drug is removed.[56,57]

Commentary

To increase our knowledge about the origin of human diseases, experimental animal models are to a certain extent irreplaceable. The models described above simulate human colorectal carcinogenesis and serve to understand the mechanisms of malignant degeneration. Chemically-induced tumors of the large bowel in rodents mimic human colorectal neoplasia in almost every respect and thus provide an acceptable base for (further) investigations.

Experiments in animal models have both supported and contradicted the epidemiological data. A solution for the contradictions requires better controlled studies in available models. It demands development of new models that will provide reliable, consistent data on which recommendations for prevention and therapy of the disease can be based.

Despite conflicting results concerning the role of sex steroids in colon carcinogenesis (Tables 1 and 2) the following facts remain to be considered—the presence of steroid receptors may be a base for therapy and could be a prognostic factor for endocrine therapy.

Table 1. Sex steroids in colon carcinogenesis

Sex steroids and where found	Reference
I. Normal intestinal epithelium	
Androgen receptors	
Castrated male rats	25
No testosterone-binding proteins in intact human colonic mucosa	58
Distal large intestine of carcinogen (DMH) treated female and male rats	30
Male rats	19
Colonic mucosa of patients with rectal and cecal tumors and with diverticulosis coli (both sexes)	22
No increase of mucosal androgen receptors following carcinogen treatment	19
Estrogen receptors	
Non-tumorous mucosa of patients with bowel cancer	5
No receptors in colonic mucosa of carcinogen (DMH) treated rats	26
Normal human colonic mucosa	59
Patients with ulcerative colitis	
Non-tumorous mucosa of patients with large bowel cancer (no significant sex differences reported)	
Progesterone receptors	
Non-tumorous colonic mucosa of carcinogen (DMH) treated male and female rats	30
Human colonic mucosa	59
II. Intestinal tumors and adjacent mucosa	
Androgen receptors	
DMH-treated male BD IX rats; negative in female rats	25
Both human colon tumors and adjacent colon mucosa	58
Male rats	26
Male > female rats	30
AOM-induced colonic rat tumors	19
Tumor tissue > adjacent mucosa	25, 30
Tumor tissue = adjacent mucosa	26
Tumor tissue < adjacent mucosa	19
Estrogen receptors	
Male rats	26
DMH-induced BD IX rat tumors	30
Human colorectal adenocarcinoma xenotransplanted onto Balb/c nude mice	33
Progesterone receptors	
Human colorectal adenocarcinoma xenotransplanted onto Balb/c nude mice	33
DMH-induced BD IX-rat tumors	30
Decrease of colon cancer incidence in DMH-treated BD IX-rats following castration (*not* in BD II-rats)	13
Increase of susceptibility to carcinogenesis in DMH treated and castrated BD IX rats after androgen application (*not* in BD II-rats)	
Decrease of colon cancer incidence in AOM-treated Fischer-344 rats after surgical castration	14
Increase of tumor incidence in AOM-treated Sprague–Dawley rats following surgical castration	19
Increase of colonic tumorigenesis in AOM-treated Sprague–Dawley rats following chemical castration (using cyproterone-acetate)	29
Decrease of colonic tumorigenesis in AOM-treated Sprague–Dawley rats following testosterone application after surgical castration	

Table 2. Response of intestinal tumors to treatment with sex steroids

Sex steroid	Reference
Androgens	
Decrease of mitotic rate in DMH-induced colonic rat tumors by castration and application of anti-androgen (*flutamide*); increase of tumor-cell proliferation following testosterone application	60
Increase of colon-tumor incidence in castrated and AOM-treated Fischer 344 rats	14

Table 2 (*cont.*)

Estrogens	
Decrease of mitotic rate in DMH-induced rat colon tumors following ovariectomy, reversible by estradiol application (not reversible by progesterone treatment: see below)	61
Growth-decrease of human colorectal adenocarcinoma xenotransplanted onto Balb/c nude mice	33
Progesterone	
No effect on mitotic rate in DMH-induced colonic rat tumors after ovariectomy	61
Growth-decrease of human colorectal adenocarcinoma xenotransplanted onto Balb/c nude mice	33

References

1. LaMont JT, O'Gorman TA. Experimental colon cancer. *Gastroenterology* 1978; **75**: 1157–69.
2. Berg JW. Can nutrition explain the pattern of international epidemiology of hormone-dependent cancers? *Cancer Res* 1975; **35**: 3345–507.
3. Lynch HT, Krush AJ, Guirgis H. Genetic factors in families with combined gastrointestinal and breast cancers. *Am J Gastroenterol* 1973; **59**: 31–40.
4. McMichael AJ, Potter JD. Reproduction, endogenous and exogenous sex hormones, and colon cancer: a review and hypothesis. *JNCI* 1980; **65**: 1201–07.
5. Alford TC, Do HM, Geelhood GW, Tsangari N, Lippman LI. Steroid hormone receptors in human colon cancers. *Cancer* 1979; **43**: 980-4.
6. Odagiri E, Jibiki K, Demura R, *et al.* Steroid receptors and the distribution of IR-carcinoembryonic antigen in colonic cancer. *Dis Colon Rectum* 1984; **27**: 787–91.
7. Laqueur GL, Mickelsen O, Whiting MG, Kurland LT. Carcinogenic properties of nuts from 'Cycas circinalis L.' indigenous to Guam. *J. Natl Cancer Inst* 1963; **31**: 919.
8. Druckrey H, Preussman R, Matzkies F, Ivankovic S. Selektive Erzeugung vor Darmkrebs bei Ratten durch 1,2-Dimethylhydrazin. *Naturwissenschaften* 1967; **54**: 285–6.
9. Druckrey H. Organospecific carcinogenesis in the digestive tract. In: Nakahara W, Takayama S, Sugimura T, Odashima S, eds. *Topics in Chemical Carcinogenesis*, Baltimore: University Park Press, 1972.
10. Gilbert JM. Experimental colorectal cancer as a model of human disease. *Ann R Coll Surg Enfl* 1987; **69**: 48–53.
11. Koch M, McPherson TA, Egedahl RD. Effect of sex and reproductive history on the survival of patients with colorectal cancer. *J. Chron Dis* 1982; **35**: 69–72.
12. Balish E, Shi CN, Croft WA, *et al.* Effects of age, sex and intestinal flora on the induction of colon tumors in rats. *J. Natl Cancer Inst* 1977; **58**: 1103–06.
13. Moon RC, Fricks CM. Influence of gonadal hormones and age on 1,2-DMH-induced colon-carcinogenesis. *Cancer* 1977; **40**: 2502–05.
14. Izbicki JR, Schmitz R, Kamran D, Izbicki W. Androgens as promoters of colon carcinogenesis. *Cancer Detect Prev* 1983; **6**: 355–62.
15. Izbicki JR, Dornschneider G, Hamilton SR, Nagelschmidt M. Sequential changes in colonic mucosal morphology and epithelial proliferation during chemically induced carcinogenesis in rats. *Dig Surg* 1988; **5**: 99–108.
16. Nigro ND. Animal model for colorectal cancer. *Prog Clin Biol Res* 1985; **186**: 161–73.
17. Bird RP, Mercer NJ, Draper HH. Animal models for the study of nutrition and human disease: colon cancer, arteriosclerosis and osteoporosis. *Adv Nutr Res* 1985; **7**: 155–86.
18. Rogers AE, Nauss KM. Rodent models for carcinoma of the colon. *Dig Dis Sci* 1985; **30**: 87–102.
19. Izbicki JR, Wambach G, Hamilton SR, *et al.* Androgen receptors in experimentally induced colon-carcinogenesis. *J Cancer Res Clin Oncol* 1986; **112**: 39–46.
20. Bucci L, Salfi R, Meraviglia F, Delric G. Hormonal receptors in colorectal cancers. *2nd Eur Conf on Clin Oncology and Cancer Nursing* 1983; **41**: 98 (abstract).
21. d'Istria M, Fasano S, Catuogno F, *et al.* Androgen and progesterone receptors in colonic and rectal cancers. *Dis Colon Rectum* 1986; **29**: 263–5.
22. Stebbings WS, Farthing MJ, Vinson JP, Northover JM, Wood RF. Androgen receptors in rectal and colonic cancer. *Dis Colon Rectum* 1986; **29**: 95–8.
23. Wobbes TH, Beex LVAM, Koenders AJM. Estrogen and progestin receptors in colonic cancer? *Dis Colon Rectum* 1984; **27**: 591–2.
24. Stebbings WSL, Farthing MJG, Vinson GP, Wood RFM. Do sex hormones affect colorectal cancer? Letters, *Br Med J Clin Res* 1985; **291**: 138.
25. Metha RG, Fricks CM, Moon RC. Androgen receptors in chemically-induced colon-carcinogenesis. *Cancer* 1980; **45**: 1085–9.
26. Krelenbaum M, Kareem AM, Fleizser D, Fazekas AG. Steroid hormone receptors in DMH induced rat colonic tumors. *Anticancer Res* 1984; **4**: 395–8.
27. Hoff MB, Chang WWL. The effect of estrogen on epithelial cell proliferation in colonic mucosa in the mouse. *Virchow's Archiv* 1981; **35**: 263–73.
28. Tutton PJM, Barkla DH. Steroid hormones as regulators of the proliferative activity of normal and neoplastic intestinal epithelial cells (review). *Anticancer Res* 1988; **8**: 451–6.
29. Izbicki JR, Hamilton SR, Wambach G, *et al.* Effects of androgen manipulations on chemically induced colonic tumors and on macroscopically normal colonic mucosa in male Sprague–Dawley rats. *Br J Cancer* 1990; **61**: 235–40.
30. Odagiri E, Jibiki K, Kato Y, *et al.* Steroid receptors in DMH-induced colon carcinogenesis. *Cancer* 1985; **56**: 2627–34.
31. Furr BJA, Jordan VC. The pharmacology and clinical uses of tamoxifen. *Pharmac Ther* 1984; **25**: 127–205.
32. Howell MA. The association between colorectal cancer and breast cancer. *J Chronic Dis* 1976; **29**: 243–61.
33. Izbicki JR, Schmitz R, Hoppen HO, *et al.* Effects of steroid hormone therapy on primarily xenotransplanted human colorectal adenocarcinomas. *J Cancer Res Clin Oncol* 1984; **108**: 345–50.
34. Eisenfeld AJ, Aten RF. Estrogen receptors and androgen receptors in the mammalian liver. *J Steroid Biochem* 1987; **27**: 1109-18.

35. Kohigashi K, Fukuda Y, Imura H. Estrogen receptors in hepatocellular carcinoma: is endocrine therapy for hepatocellular carcinoma likely to be effective? *Gastroenterol Jpn* 1987; **22**: 322-30.
36. Lax ER. Mechanisms of physiological and pharmacological sex hormone action on the mammalian liver. *J Steroid Biochem* 1987; **27**: 1119-28.
37. Marschke KB, Koritnik DR. Estrogen and androgen receptors in the liver of cynomolgus monkeys (Macaca fascicularis). *J Steroid Biochem* 1987; **26**: 443-50.
38. Winborn WB, Sheridan PJ, McGill HC Jr. Sex steroid receptors in the stomach, liver, pancreas, and gastrointestinal tract of the baboon. *Gastroenterology* 1987; **92**: 23-32.
39. Manni A, Santen RJ, Boucher A, *et al.* Hormone stimulation and chemotherapy in advanced prostate cancer: preliminary results of a prospective controlled clinical trial. *Anticancer Res* 1985; **5**: 161-5.
40. Meikle AW, Smith JA, Stringham JD. Estradiol and testosterone metabolism and production in men with prostatic cancer. *J Steroid Biochem* 1989; **33**: 19-24.
41. Tejura S, Rodgers GR, Dunion MH, *et al.* Sex-steroid receptors in the diethylnitrosamine model of hepatocarcinogenesis: modifications by gonadal ablation and steroid replacement therapy. *J Mol Endocrinol* 1988; **3**: 220-37.
42. Wilkinson ML. Sex-steroids and primary hepatocellular carcinoma. *Anticancer Res* 1987; **7**: 1071-7.
43. Andren-Sandberg A. Androgen influence on exocrine pancreatic cancer. *Int J Pancreatol* 1989; **4**: 363-9.
44. Lhoste EF, Roebuck BD, Brinck-Johnsen T, Longnecker DS. Effect of castration and hormone replacement on azaserine-induced pancreatic carcinogenesis in male and female Fischer rats. *Carcinogenesis* 1987; **8**: 699-703.
45. Pousette A, Fernstad R, Skoldefors H, *et al.* Analysis of an estrogen binding macromolecule in human pancreas by radioimmunoassay. *J Steroid Biochem* 1987; **26**: 439-42.
46. Saydjari R, Singh P, Affini B, *et al.* The isolation and characterization of estrogen binding proteins in the pancreas of male and female hamsters. *J Steroid Biochem* 1988; **29**: 41-5.
47. Sumi C, Longnecker DS, Roebuck BD, *et al.* Inhibitory effects of estrogen and castration on the early stage of pancreatic carcinogenesis in Fischer rats treated with azaserine. *Cancer Res* 1989; **49**: 2332-6.
48. Pousette A, Fernstad R, Haggmark A, *et al.* The estrogen binding protein in human pancreas: concentration in subcellular fractions of normal pancreatic tissue, in duodenal juice during pancreatic stimulation and in peripheral serum in normal and pathological conditions. *J Steroid Biochem* 1988; **29**: 423-7.
49. Jacobs M. Selenium inhibition of 1,2-dimethylhydrazine-induced colon-carcinogenesis. *Cancer Res* 1983; **43**: 1646.
50. Cook MG, McNamarra P. Effect of dietary vitamin E on DMH-induced colonic tumors in mice. *Cancer Res* 1980; **40**: 1329–31.
51. Colacchio TA, Menoli VA. Chemoprevention of colorectal neoplasms. Ascorbic acid and β-carotene. *Arch Surgery* 1986; **121**: 1421–4.
52. Fiala ES. Inhibition of carcinogen metabolism and action by disulfiram, pyrazole, and related compounds. In: MS Zedeck, M Lipkin, eds. *Inhibition of Tumor Induction and Development*, New York: Plenum Press, 1981: 23.
53. Reddy BS, Maruyama G. Dose-related inhibition of colon-carcinogenesis by dietary piroxicam, a non-steroidal anti-inflammatory drug, during different stages of rat colon tumor-development. *Cancer Res* 1987; **47**: 5340–6.
54. Ferreira SH, Vane JR. New aspects of mode of action of non-steroidal anti-inflammatory drugs. *Annu Rev Pharmacol* 1974; **14**: 57.
55. Wattenberg LW. Inhibition of DMH-induced neoplasia of the large intestine by disulfiram. *J Natl Cancer Inst* 1975; **54**: 1005–06.
56. Narisawa T, Sato M, Tani M, Kudo T, Takahashi T. Inhibition of development of methylnitrosurea-induced rat colon tumor by indomethacin. *Cancer Res* 1981; **41**: 1954–7.
57. Pollard M, Luckert P. Prolonged antitumor effect of indomethacin on autochthonusintestinal tumors in rats. *J Natl Cancer Inst* 1983; **70**: 1103–05.
58. Sani BP, Banerjee CK, Peckham JC. The presence of binding proteins for retinoic acid and dihydrotestosterone in murine and human colon tumors. *Cancer* 1980; **46**: 2421–9.
59. Marugo M, Molinari F, Fazzuoli L, *et al.* Estradiol and progesterone receptors in normal and pathologic colonic mucosa in humans. *J Endocrinol Invest* 1985; **8**: 117–9.
60. Tutton PJM, Barkla DH. Differential effects of estrogenic hormones on cell proliferation in the colonic crypt epithelium and in colonic carcinoma of rats. *Anticancer Res* 1982; **2**: 199–201.
61. Tutton PJM, Barkla DH. The influence of androgens, anti-androgens, and castration on cell proliferation in the jejunal and colonic crypt epithelia, and in DMH-induced adenocarcinoma of rat-colon. *Virchows-Arch* 1982; **38**: 351–5.

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