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Review Article

A review of experimental models in colorectal carcinogenesis☆**Vanessa Foresto Machado, Marley Ribeiro Feitosa*, Jose Joaquim Ribeiro da Rocha, Omar Féres**

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ABSTRACT

Colorectal cancer is the leading cause of malignancy of the gastrointestinal tract. A better understanding of the molecular and cellular changes that lead to the disease is necessary to develop early diagnosis and optimal treatment modalities. Rodent models are rapid, reproducible and exhibit an adenoma-carcinoma sequence similar to that found in humans. The objective of this manuscript is to review the most common chemical carcinogens used to induce experimental tumors and the usual methods of evaluation.

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Modelos experimentais de carcinogênese colorretal

RESUMO

O câncer colorretal é a principal neoplasia maligna do trato gastrointestinal. Um melhor entendimento dos processos moleculares e celulares é necessário para o desenvolvimento de estratégias que permitam um diagnóstico precoce e um tratamento mais eficaz. Modelos que utilizam roedores são rápidos, reprodutíveis e permitem o estudo da sequência adenoma-carcinoma de forma similar a encontrada em humanos. O objetivo desse manuscrito é revisar os principais modelos de carcinogênese química e os métodos mais usuais para avaliação dos resultados.

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Introduction

Colorectal cancer (CRC) is the most common gastrointestinal malignancy. Worldwide, CRC is the third most common cancer in men and the second in women. In Brazil, a total of 32,000 new cases of CRC are expected in 2015.^{1,2}

Colorectal malignant neoplasms are characterized by an excessive and uncontrolled growth of abnormal cells originating from any part of the colon. Unlike benign lesions, cancer is morphologically different from the normal tissue of origin, have the ability to invade and destroy surrounding tissues, metastasis to distant organs and, if left untreated, will lead to death.

The factors involved in the origin and progression of colon tumors have been an area of increasing interest. Since the first experiment of colon tumors induction, more accurate models and various substances with carcinogenic activity have been studied. Carcinogen-induced colon cancer, have proved to share many similarities with human tumors and significantly contributed to our actual understanding regarding cancer pathogenesis.³

The ideal experimental carcinogen should induce neoplasms in the colon, however, most agents lack specificity (e.g. 1,2-dimethylhydrazine, that may induce liver tumors). Reproducibility is another important feature that may be hampered by the heterogeneous species and lineages of animals, each with different patterns of drug response. Moreover, there is no standardization of dose and time of tumor development, to the various studied drugs.

Rodents are widely accepted models of colorectal carcinogenesis because of their similarity with humans. Advantages include rapid, reproducible tumor induction and the possibility to study the adenoma-carcinoma sequence.⁴

Natural colorectal carcinogenesis factors may be divided into two main categories: those related to genetic or environmental factors.

Genetic factors

The importance of oncogenes and suppressive genes are widely recognized. Early genetic changes include chromosome 5 (APC) and *ras* mutations while allelic losses in chromosomes 17 (*p53*) and 18 (*DCC*) happen late in the adenoma-carcinoma sequence.⁵ A schematic genetic model of the adenoma-carcinoma sequence is shown in Fig. 1.

Environmental factors

Diet plays an important role on carcinogenesis and, in some countries with higher CRC prevalence, an attributed risk of 50% is estimated.⁶ Geographic variations in CRC incidences and studies with immigrant populations suggest that lifestyle factors including poor diet, physical inactivity and alcohol consumption are associated to an increased risk of CRC. While increased red meat consumption may be harmful, omega-3, vitamin D, phenolic compounds and a fiber-rich diet may lower the risk of CRC. Regular physical activity may lower the risk in 24%; on the other hand, obesity may increase it in 19%. Although moderate to high doses of alcohol have proven to

be deleterious, some studies have observed a protective effect when light doses are consumed.^{7,8}

Methods of chemical carcinogenesis

There are two types of chemical agents: (1) direct agents, that do not need metabolization by the organism to have the deleterious effect and (2) indirect agents, which are not active unless enzymatic reactions convert them to an active form.⁹

1,2-Dimetilhidrazine (DMH)

DMH is the metabolic precursor of methylazoxymethanol (MAM). It is the oldest and the most used carcinogen to induce tumors in rats. Uptake of DMH is three times greater in the colon cells compared to the enterocytes. The carcinogenic effect may be obtained after a single injection or via a series of weekly injections.^{10,11} The malignant lesion originates from the non-dysplastic mucosa and becomes evident after 4–30 weeks after administration of the drug. Even after the administration of small doses of the drug, up to 80% of the treated mice can develop adenocarcinoma.¹²

Azoxymethane (AOM)

AOM is a metabolite of DMH. Its carcinogenesis mechanism is attributed to *c-fos* overexpression, reduced expression of *c-myc* and *k-ras* mutation. These changes are similar to those observed in spontaneous carcinogenesis in humans.¹³ Compared to DMH, AOM is more potent and requires fewer reactions to its activation, which makes a better option. It is activated in the liver by N-oxidation, and produces essential reactive compounds for chemical carcinogenesis (methylazoxymethanol and methyl-diazoxide), which are brought to the colon through the bloodstream or bile as conjugated glucuronide.

Heterocyclic amines (HAs)

Among the HAs, 2-amino-3-methylimidazo [4,5-f] quinolone (IQ) and 2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine (PhIP) have gained attention after proved to be highly mutagenic and tumorigenic in rodents. They appear to have target-organ specificity and can induce malignancies of the colon, prostate and mammary glands. Creatinine, sugars and amino acids from red meat and fish are the precursors of IQ. The final active compound is formed hepatic metabolization. Incidence of induced colon cancer may reach 28% after administering a diet rich in IQ and PhIP, for 52 weeks. They became not only an interesting model of carcinogenesis but can also help investigation of chemoprotection effect against cancer, of some substances.¹⁴

Aromatic amines

In 1941, *Lorenz et al.* observed the induction of intestinal tumors in mice fed with 1,2,5,6-dibenzanthracene and 20-methyleholanthrene. This effect was also studied by *Walpole*

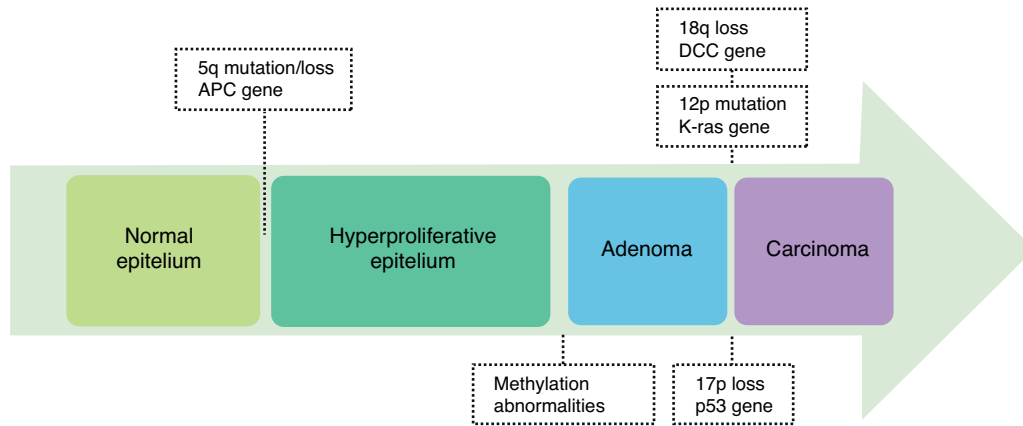


Fig. 1 – The adenoma-carcinoma sequence. Adapted from Vogelstein.⁵

et al. who noticed colon tumor induction in mice, after subcutaneous administration of 3,2'-dimethyl-4-aminobiphenyl (DMAB).

After 20 weeks of 50 mg/kg subcutaneous injections of DMAB in male F344 rats multiple adenomas and adenocarcinomas were induced in 30% of animals fed with low-fat diet and in 74% of the animals with high-fat diet.

This model has two disadvantages: (1) the need for multiple injections of DMAB and (2) low specificity, since tumors may be induced in other tissues such as adenocarcinoma of mammary glands, sarcoma of salivary glands, squamous cell carcinoma of the ear and skin, gastric squamous cell papilloma, sarcoma, lymphoma and urothelial carcinoma of the bladder.^{15,16}

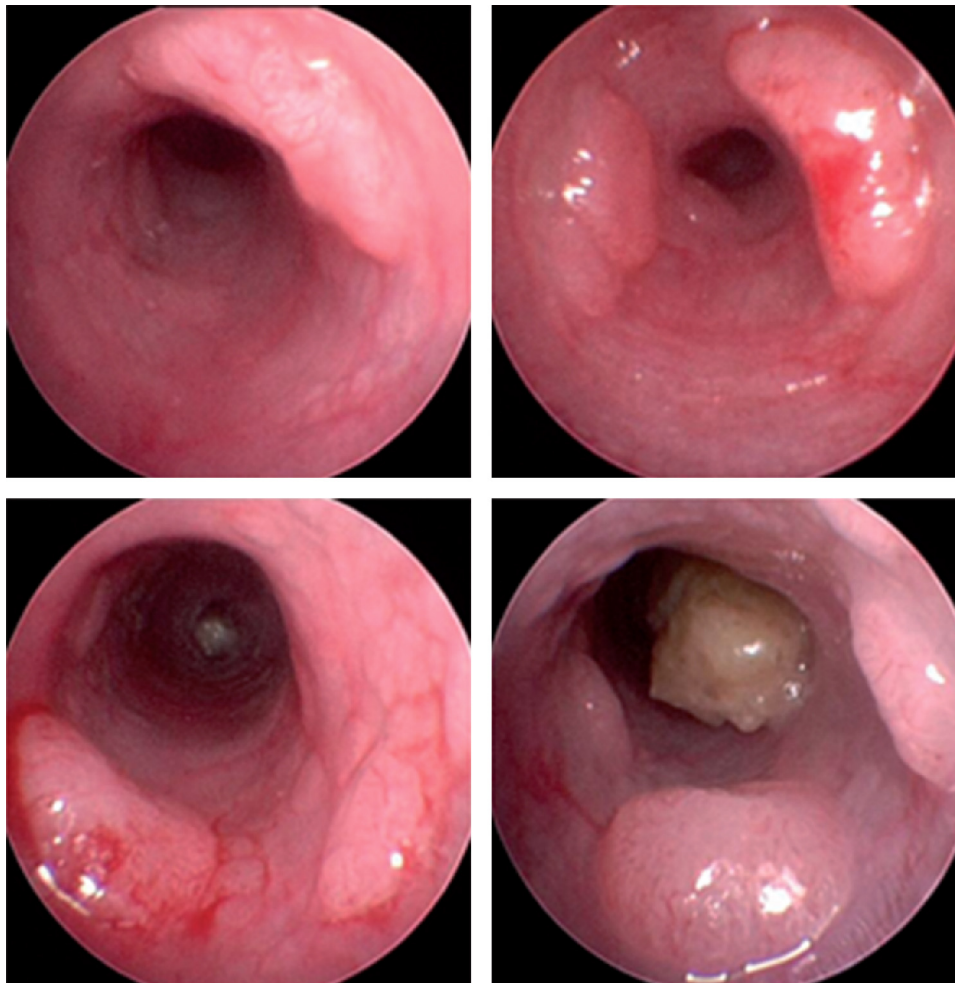


Fig. 2 – Endoscopic visualization of AOM-induced tumors in mice. Adapted from Neufert *et al.*²⁷

Alkylnitrosamines

Alkylnitrosamines such as methylnitrosourea (MNU) and N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) are direct-acting carcinogens with high affinity and methylation capacity of specific DNA regions. Rectal instillation of such compounds induce sessile and polypoid colorectal tumors in rodents. Doses of 1–3 mg/week of MNNG instilled per rectum, for 20 weeks, induced tumors in 100% of F344 female rats. The main disadvantage of this model is the difficulty to instill a precise amount of the drug per rectum.¹⁷

Evaluation of experimental models

Histological method

Optical microscopy can assess cellular changes including the development of aberrant crypt foci (ACF) that may be considered a preneoplastic event. The development of ACF is considered an early marker of a future CRC.¹⁸ Tumors may be classified as adenomas or adenocarcinomas. Optical microscopy may also evaluate the differentiation grade and the level of penetration through the intestinal wall (Tis, T1, T2, T3 e T4).

Immunohistochemistry

The method consists in associating specific histochemical dyes to an antigen-antibody reaction, which can increase the identification rate of tumors. Different antigens can be used in this method:

Metallothionein (MT): MTs are low-molecular-weight proteins, rich in cysteine residues, mainly expressed in the liver, kidney, intestine and brain. They play an important role in protecting against oxidative stress by binding metals such as zinc and copper. Elevated MT levels can be found in cancerous tissues with high proliferation rates, since they can provide zinc and copper ions for the metabolism of nucleic acids, protein synthesis, and other metabolic processes. MT has become an important biomarker of preneoplastic lesions of the colon, where it is predominantly expressed by mutated crypt stem cells.¹⁹

Gamma-H2AX: histones are proteins that compact and order DNA into nucleosomes. They play an important role on DNA transcription, replication and repair. H2AX originates from H2A phosphorylation after DNA damage and is responsible for recruitment of repair proteins. H2AX is, therefore, an important DNA damage biomarker.²⁰

Vascular endothelial growth factor (VEGF): angiogenesis is an essential mechanism for the growth and development of CRC. VEGF is closely associated with neovascular formation in colorectal neoplasia. A hypoxic tumor microenvironment is critical to the neoplastic process and a strong stimulus for the production of VEGF via hypoxia-inducible factor 1 α (HIF-1 α).^{21,22}

Hypoxia-inducible factor 1 α : HIF-1 α is a dimeric transcription factor, which is the key to cellular homeostasis under hypoxia condition by regulating the expression of various genes involved in cancer biology.²³

Anti-COX2: early indications of COX-2 role in CRC development came from the observation that patients with Gardner's syndrome treated with non-steroidal anti-inflammatory drugs had reduced numbers of adenomas. Indeed, elevated levels of COX-2 may be found in some adenomas and in adenocarcinomas. The tumorigenic effect of COX-2 may be attributed to the production of prostaglandin E (PGE2). A disruption in the COX-2/PGE2 cascade appears to affect colorectal tumorigenesis by promoting the maintenance and progression of tumor, increased metastasis rate and even participating in tumor initiation.²⁴

Cytokine assay

The immune response may contribute to neoplastic transformation. Some studies have shown that inhibition of proinflammatory cytokines, such as tumor necrosis factor alpha (TNF- α), may lead to reduction of preneoplastic lesions. On the other hand, production of proinflammatory cytokines and chemokines by macrophages and mast cells, for example, may stimulate other cells to produce cytokines with anti-inflammatory activity, such as interleukin-10 (IL-10), which may contribute to tumor-associated immunosuppression.²⁵

Endoscopy

High-resolution endoscopic devices can assess mucosal changes consistent with preneoplastic lesions or cancer. Endoscopic aspects are similar to humans. Vegetating and ulcerated lesions can be found (Fig. 2). Serial biopsies are required to diagnose the nature of the lesions. Scores that take the amount and size of tumors into account should be used to obtain uniform evaluations.^{26,27}

Conclusion

Experimental models allow us to study the early stages of carcinogenesis including the adenoma-carcinoma sequence. A better understanding of the biochemical and cellular changes that determine carcinogenesis can help the development of new methods of diagnosis, as well as better and more effective treatment.

Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES

1. [Estimativa 2014: Incidência de Câncer no Brasil. Rio de Janeiro: INCA; 2014.](#)
2. [Stewart BW, Wild CP. World cancer report 2014. Lyon; 2014.](#)
3. [Taketo MM, Edelmann W. Mouse models of colon cancer. Gastroenterology. 2009;136:780–98.](#)
4. [Balmain A, Harris CC. Carcinogenesis in mouse and human cells: parallels and paradoxes. Carcinogenesis. 2000;21:371–7.](#)
5. [Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, et al. Genetic alterations during colorectal-tumor development. N Engl J Med. 1988;319:525–32.](#)

6. Kune GA, Bannerman S, Watson LF. Attributable risk for diet, alcohol, and family history in the Melbourne Colorectal Cancer Study. *Nutr Cancer*. 1992;18:231-5.
7. Cardoso JF, Cohen C, Jordao AA Jr, Vannucchi H, Garcia SB, Zucoloto S. Light and moderate doses of ethanol in chemical carcinogenesis of the colon in rats. *Nutr Cancer*. 2011;63:1029-35.
8. Weng CJ, Yen GC. Chemopreventive effects of dietary phytochemicals against cancer invasion and metastasis: phenolic acids, monophenol, polyphenol, and their derivatives. *Cancer Treat Rev*. 2012;38:76-87.
9. Tanaka T. Colorectal carcinogenesis: review of human and experimental animal studies. *J Carcinog*. 2009;8:5.
10. Oliveira EC, Leite MS, Miranda JA, Andrade AL, Garcia SB, Luquetti AO, et al. Chronic *Trypanosoma cruzi* infection associated with low incidence of 1,2-dimethylhydrazine-induced colon cancer in rats. *Carcinogenesis*. 2001;22:737-40.
11. Gois E Jr, Daniel RA, Parra RS, Almeida AL, Rocha JJ, Garcia SB, et al. Hyperbaric oxygen therapy reduces COX-2 expression in a dimethylhydrazine-induced rat model of colorectal carcinogenesis. *Undersea Hyperb Med*. 2012;39:693-8.
12. Rodrigues MA, Silva LA, Salvadori DM, De Camargo JL, Montenegro MR. Aberrant crypt foci and colon cancer: comparison between a short- and medium-term bioassay for colon carcinogenesis using dimethylhydrazine in Wistar rats. *Braz J Med Biol Res*. 2002;35:351-5.
13. Caderni G, Femia AP, Giannini A, Favuzza A, Luceri C, Salvadori M, et al. Identification of mucin-depleted foci in the unsectioned colon of azoxymethane-treated rats: correlation with carcinogenesis. *Cancer Res*. 2003;63:2388-92.
14. Rosenberg DW, Giardina C, Tanaka T. Mouse models for the study of colon carcinogenesis. *Carcinogenesis*. 2009;30:183-96.
15. Walpole AL, Williams MH, Roberts DC. The carcinogenic action of 4-aminodiphenyl and 3:2'-dimethyl-4-amino-diphenyl. *Br J Ind Med*. 1952;9:255-63.
16. Lorenz E, Stewart HL. Intestinal carcinoma and other lesions in mice following oral administration of 1,2,5,6-dibenzanthracene and 20-methylcholanthrene. *J Natl Cancer Inst*. 1940;1:17-40.
17. Maurin N, Forgue-Lafitte ME, Levy P, Zimmer A, Bara J. Progression of tumors arising from large ACF is associated with the MUC5AC expression during rat colon MNNG carcinogenesis. *Int J Cancer*. 2007;120:477-83.
18. Pretlow TP, Barrow BJ, Ashton WS, O'Riordan MA, Pretlow TG, Jurcisek JA, et al. Aberrant crypts: putative preneoplastic foci in human colonic mucosa. *Cancer Res*. 1991;51:1564-7.
19. Bruewer M, Schmid KW, Kriegelstein CF, Senninger N, Schuermann G. Metallothionein: early marker in the carcinogenesis of ulcerative colitis-associated colorectal carcinoma. *World J Surg*. 2002;26:726-31.
20. Kuo LJ, Yang LX. Gamma-H2AX – a novel biomarker for DNA double-strand breaks. In vivo (Athens, Greece). 2008;22:305-9.
21. Stacker SA, Caesar C, Baldwin ME, Thornton GE, Williams RA, Prevo R, et al. VEGF-D promotes the metastatic spread of tumor cells via the lymphatics. *Nat Med*. 2001;7:186-91.
22. Daniel RA, Cardoso VK, Gois E Jr, Parra RS, Garcia SB, Rocha JJ, et al. Effect of hyperbaric oxygen therapy on the intestinal ischemia reperfusion injury. *Acta Cir Bras*. 2011;26:463-9.
23. Chen H, Feng J, Zhang Y, Shen A, Chen Y, Lin J, et al. Pien Tze Huang inhibits hypoxia-induced angiogenesis via HIF-1 α /VEGF-A pathway in colorectal cancer. *Evid Based Complement Alternat Med*. 2015;2015:454279.
24. Greenhough A, Smartt HJ, Moore AE, Roberts HR, Williams AC, Paraskeva C, et al. The COX-2/PGE2 pathway: key roles in the hallmarks of cancer and adaptation to the tumour microenvironment. *Carcinogenesis*. 2009;30:377-86.
25. Klampfer L. Cytokines, inflammation and colon cancer. *Curr Cancer Drug Targets*. 2011;11:451-64.
26. Becker C, Fantini MC, Wirtz S, Nikolaev A, Kiesslich R, Lehr HA, et al. In vivo imaging of colitis and colon cancer development in mice using high resolution chromoendoscopy. *Gut*. 2005;54:950-4.
27. Neufert C, Becker C, Tureci O, Waldner MJ, Backert I, Floh K, et al. Tumor fibroblast-derived epiregulin promotes growth of colitis-associated neoplasms through ERK. *J Clin Investig*. 2013;123:1428-43.