ANIMAL MODELS FOR COLORECTAL CANCER

Models animais de carcinogênese colorectal

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ABSTRACT – Introduction: Colorectal cancer is a very frequent sort of neoplasm among the population, with a high mortality rate. It develops from an association of genetic and environmental factors, and it is related to multiple cell signaling pathways. Cell cultures and animal models are used in research to reproduce the process of disease development in humans. Of the existing animal models, the most commonly used are animals with tumors induced by chemical agents and genetically modified animals. Objective: To present and synthesize the main animal models of colorectal carcinogenesis used in the research, comparing its advantages and disadvantages. Method: This literature review was performed through the search for scientific articles over the last 18 years in PubMed and Science Direct databases, by using keywords such as “animal models”, “colorectal carcinogenesis” and “tumor induction”. Results: 1,2-dimethylhydrazine and azoxymethane are carcinogenic agents with high specificity for the small and large intestine regions. Therefore, the two substances are widely used. Concerning the genetically modified animal models, there is a larger number of studies concerning mutations of the APC, p53 and K-ras genes. Animals with the APC gene mutation develop colorectal neoplasms, whereas animals with p53 and K-ras genes mutations are able to potentiate the effects of the APC gene mutation as well as the chemical inducers. Conclusion: Each animal model has advantages and disadvantages, and some are individually efficient as to the induction of carcinogenesis, and in other cases the association of two forms of induction is the best way to obtain representative results of carcinogenesis in humans.

INTRODUCTION

Cancer is a sort of disease that can reach most of the organs and tissues of the human body. It is characterized primarily by the disordered growth of cells, often able to metastasize to other regions of the body. According to Petit et al.¹⁹, the disease is associated with both genetic factors, inherent to each individual, and environmental factors.

Colorectal cancer (CRC) is the 4th most frequent neoplasm among the world’s population, accounting for 694,000 of the 8.2 million cancer deaths in 2012.²⁰ It is a very aggressive type of cancer that has a high potential to spread to other organs. It develops in 90-95% of cases due to environmental factors²¹-²⁴ and it is related to multiple signal transduction cascades, important for different types of biological response such as angiogenesis, apoptosis and cell proliferation.²⁵-²⁹

CRC may be hereditary or sporadic, accounting for 80% of all patients affected by the disease. The hereditary form is related to two familial syndromes, familial adenomatous polyposis, in which the appearance of multiple intestinal polyps,
and hereditary nonpolyposis colorectal cancer is observed. Individuals who develop familial adenomatous polyposis have a mutation in the APC (adenomatous polyposis of the colon) tumor suppressor gene, whereas those who develop hereditary nonpolyposis colorectal cancer have mutations in genes involved in DNA repair and mismatch repair (MMR) genes. The sporadic form, in turn, is related to inflammatory bowel processes such as Crohn’s disease and ulcerative colitis, as well as to eating habits such as red meat consumption and low fiber intake.

For studies related to the development, treatment and prevention of colon and rectum tumors, animal models or cell cultures, which are representative of a carcinogenic situation in humans, are used. However, in spite of all the ethical conflicts involved in animal experimentation, one of the major disadvantages of cell culture, compared to animal models, is the inability to reproduce metastatic and angiogenesis situations.

For research purposes, the two major animal models of colorectal carcinogenesis are colorectal tumors induced by chemical or environmental agents in rodents, which represent sporadic CRC, and genetically modified mice, which represent the hereditary familial adenomatous polyposis and hereditary non-polyposis colorectal cancer syndromes.

This review aims to present and synthesize the main animal models of colorectal carcinogenesis used in the research, comparing its advantages and disadvantages.

**METHOD**

For the development of this review, a search for scientific articles over the last 18 years in the PubMed and Science Direct databases was carried out, using keywords such as “animal models”, “colorectal neoplasias” and “induced colon cancer”. The search resulted in 89 articles, of which 31 were selected as the most relevant for this review.

**RESULTS**

Colorectal cancer induced by exogenous agents

According to Andrade et al., there are several factors capable of increasing the chances of developing colorectal cancer, among them the intake of foods rich in fat, and with low fiber content. Thus, the eating habits of an individual can directly influence the occurrence of this neoplasia. Newmark et al. observed that 25% of the animals fed for two years on this diet had invasive adenocarcinomas in small bowel, cecum and proximal colon regions; and that another group, fed with a diet enriched with calcium and vitamin D, did not present intestinal lesions. In addition, Yang et al. observed that 75% of the animals, treated for a year and a half with the same lipid diet, had the APC-/- mutation and 57% the Muc2-/- mutation, which are important in CRC development. However, only 27% of them had alterations in the intestinal mucosa. The two studies showed that diet can directly influence the appearance of colorectal neoplasias, as it promotes reprogramming of intestinal cells, thus representing the spontaneous colorectal cancer model in humans. However, although the authors observed different intestinal neoformations in the animals treated with the hyper caloric diet, it was not possible to evaluate which mutations were responsible for the development of the tumors. Also, the diet may not be considered the best model of colorectal carcinogenesis, since the percentage of animals that develop the neoplasia is small, and the time for this to occur is long.

Besides the diet, chemical substances with carcinogenic potential are also used. The two most commonly used tumor inducers in animal models for induction of sporadic CRC are azoxymethane (AOM), which is a direct inducer, and 1,2 dimethylhydrazine (DMH), which is an indirect inducer of carcinogenesis. They are able to represent the mechanisms of development of CRC that occur naturally in humans, being very useful models in studies that aim to study chemopreventive and chemotherapeutic effects of other substances.

DMH is a pro-carcinogenic agent for colon cancer, it is activated in the liver and transported to the intestine by bile and blood. Its use promotes the production of free radicals, which are responsible for causing oxidative damage to the DNA of colon and liver cells.

According to Burlamaqui et al., AOM is an active metabolite of DMH also used for tumor induction in rodents. It mainly affects organs such as the liver, the lungs and the colon, and the lesions found are directly proportional to time of exposure and the dose administered.

In 2014, Lahouar et al. using AOM as an inducer, observed that animals in the AOM group had significantly more aberrant crypts, which are pre-neoplastic lesions, than the animals in the control group. In addition, they also noticed the appearance of inflammatory lesions and histological changes in the hepatic and pulmonary tissues of the animals. By 2015, Yu et al., in addition to observing pre-neoplastic lesions in mice treated with AOM, also observed a 51% and 46% increase in pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-a) and interleukin 6 (IL-6), respectively.

Aachary et al. observed that animals treated with DMH developed outbreaks of aberrant crypts and also presented alterations in the intestinal microbiota. When compared to the control group, they showed a reduction in the number of Bifidobacteria and an increase in E. coli and C. perfringens species.

Umesalma and Sudhandiran demonstrated that animals treated with DMH showed an increased expression of inflammatory markers cyclooxygenase 2 (cox-2) and IL-6. The control group presented 0.6% and 1.4% of cells expressing the two markers, respectively, and the DMH group presented 1.2% and 3.5% expressions.

DMH was also effective in CRC induction in a study by Youssef et al., which detected about 100.5 aberrant crypts foci in the 10 animals that received the inducer, and a total of 20 colorectal tumors in these same individuals, whereas the animals in the control group had neither pre-neoplastic lesions nor neoformations.

Both substances are used for CRC induction in rodents. However, there is controversy over which of the two is the most effective in tumor induction. Burlamaqui et al. stated that AOM is a more potent inducer than DMH because it is activated faster in the body. However, in a comparative study done by Jucá et al., it was observed that the induction by DMH promoted the appearance of dysplasias in mild, moderate and severe degree, in addition to carcinomas in situ, whereas the induction by AOM promoted only moderate dysplasia in the colon of the animals.

Since AOM is an active metabolite of DMH, the mechanism of induction of carcinogenesis promoted by the two substances is very similar. In the studies mentioned, the formation of aberrant crypts foci and intestinal inflammatory processes was observed in the animals treated with both DMH and AOM. However, DMH seems to be a more effective agent because in addition to the pre-neoplastic and inflammatory lesions, it also promoted the development of intestinal adenomas and carcinomas. Thus, from the models induced by exogenous agents it can be concluded that all agents, diet, AOM and DMH are able to induce CRC. But the diet proves to be a slow and low-efficiency model; the AOM is an inducer of mainly aberrant crypts foci and inflammation, and
DMH is an inducer of colorectal tumors in more advanced stages, showing to be the most efficient model.

**Genetically modified animals**

Many genes are involved in colorectal carcinogenesis, including tumor suppressors APC, DCC (deleted in colorectal cancer), p53 (gene encoding tumor protein p53) and MCC (mutated in colorectal cancer), the oncogenes K-ras(Kirsten rat sarcoma viral oncogene homolog), SRC(proto-oncogene SRC) and C-myc (homologous oncogene viral myelocytomatosis), the DNA repair genes hMSH2 (mutS 2 homologue), hMSH6 (mutS 6 homologue), hMLH1 (mutL 1 homologue), hPMS1 (BstNIproline rich protein subfamily 1) and hPMS2 (BstN1 proline rich protein subfamily 2), in addition to CD44 genes (gene encoding CD44 molecule) and COX-2 (cytochrome C oxidase, subunit 2). Each of them acts differently for the development of colorectal neoplasms, and mutations in two or more of these genes are often related to the malignancy profile of the tumors.

Thus, there are innumerable genetically modified animal models that have been developed from the knowledge about the genetic factors involved in the development of the disease. However, only the models whose mutations are most frequent in sporadic CRC in humans (APC> 70%, p53> 60% and K-ras> 40%) and in hereditary familial adenomatous polyposis(APC) and hereditary nonpolyposis colorectal cancer(MMR) will be described next.

APCmm animals are genetically modified that have a mutation in the APC gene. The acronym "mm" means multiple intestinal neoplasms, and this is an autosomal dominant mutation, which in homozygous conditions is lethal to animals. Animals that are heterozygous for the mutation develop important anemic conditions at 60 days of life and develop tumors in the large and small intestine. As in familial adenomatous polyposis cases, APCmm animals also develop colorectal adenomas, but they die at 120 days of life.

Knockout animals for p53 gene rarely develop colorectal tumors. Reed et al. reported in 2008 that p53 knockout animals did not develop colorectal neoplasias; however, the association of APCmm and p53 knockout mutations promoted an increase in aberrant crypts foci number when compared to APCmm animals. In addition, in 2008 Hu et al. reported that an association of p53 knockout animals with tumor inducer AOM was efficient in inducing carcinogenesis in the colon of the animals, and also to potentiate the action of the AOM. The same could be observed by Sakai et al.

| Animal Model | CRC type | Advantages | Disadvantages | References |
|--------------|----------|------------|---------------|------------|
| Western Diet | Sporadic | Induction of carcinogenesis in small intestine, cecum and proximal colon. | Dietary mutations have not been described yet. Few animals develop neoplastic lesions and the time of development is rather long. | 17, 28 |
| DMH | Sporadic | Capacity to induce metastasis. Induction of adenomas and adenocarcinomas. High degree of specificity for intestine. | Indirect inducer. Promotes liver toxicity. | 23 |
| AOM | Sporadic | Direct inducer. High degree of specificity for intestine. | More expensive than DMH. Promotes liver toxicity. | 4 |
| APC Sporadic and Hereditary (PAF) | Good for study of hereditary CRC. | | Animals die within 120 days. | 27 |
| PS3 Sporadic | It potentiates the action of other genes or tumor inducers. | Inhibition of gene expression alone was not able to induce colorectal carcinogenesis. | | 21 |
| K-ras Sporadic | Hyperplasia and presence of aberrant crypts. It potentiates tumorigenesis of other mutations. | They do not develop tumors, only pre-neoplastic lesions. Association with other mutations and/or inducers is required. | | 9, 15 |
| MMR Hereditary (HNPCC) | Good for study of hereditary CRC (HNPCC). | | Most tumors are in the small intestine. | 7, 15 |
DISCUSSION

Animal models are important for studies of the development and pathogenesis of colorectal tumors, as well as for the evaluation of possible risk factors, preventive agents and treatments. As shown, some individual models are representative of CRC in humans and considered to be good animal models for this type of study. However, it is noted that the association of at least two methods of colorectal carcinogenesis induction is required for most models. This is due to the fact that colon cancer is a multifactorial disease that develops by the presence of multiple factors, both genetic and environmental.

CONCLUSION

Each animal model has advantages and disadvantages, and some models are individually efficient as to the induction of carcinogenesis, and in other cases the association of at least two models of colorectal carcinogenesis induction is required for study. However, it is noted that the association of at least two methods of colorectal carcinogenesis induction is required for most models. This is due to the fact that colon cancer is a multifactorial disease that develops by the presence of multiple factors, both genetic and environmental.

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