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Obesity Strongly Promotes Growth of Mouse MC38 Colon Cancer in an Orthotopic-syngeneic C57BL/6 Mouse Model

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Abstract. *Background/Aim: Obesity is a major risk factor for colorectal cancer. The MC38 mouse colon-cancer cell line is a versatile syngeneic model of colon cancer in C57BL/6 mice. In the present study, the influence of a high-fat diet (HFD) on the growth of the MC38 mouse colon-cancer cell line was examined in an orthotopic-transplantation syngeneic model in C57BL/6 mice. Materials and Methods: Five 6-week-old C57BL/6 male mice were fed a control diet (CD, 6.5% fat) or HFD (34.3% fat) for eight weeks. Then, a 2 mm^3 fragment of a subcutaneous MC38 tumor was attached to the surface of the cecum of C57BL/6 mice with a single stitch using a 7-0 suture to establish an orthotopic-transplantation model. Each group continued their initial diet for 17 days. Results: The HFD group had more than twice the tumor volume and tumor weight than the CD group (p=0.021 and p=0.014, respectively). Conclusion: HFD-induced obesity strongly increased MC38 colon-cancer progression in a C57BL/6 orthotopic-transplantation mouse model. The present study emphasizes the detrimental effect of obesity on colon-cancer progression.*

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Key Words: Colon cancer, progression, orthotopic mouse model, MC38, syngeneic obesity, high fat diet.

Colorectal cancer is one of the most frequent causes of cancer death in the United States (1). Obesity is a risk factor for colorectal cancer. Higher body-mass index or waist-circumference scores increase the risk of colorectal cancer, as shown in a meta-analysis of prospective studies (2, 3). It is therefore critical to investigate the relationship between obesity and colon cancer. High-fat diet (HFD)-induced obesity promoted tumor growth in colon cancer, breast cancer, and melanoma in subcutaneous-tumor mouse models (4, 5).

Tumors implanted subcutaneously do not reflect the native tumor microenvironment (TME). Orthotopic cancer mouse models are more patient-like regarding their tumor microenvironment (6-8).

The aim of the present study was to establish a syngeneic orthotopic transplantation model of the MC38 mouse colon-cancer cell line using HFD-induced obese mice in order to investigate the effect of obesity on colon-cancer progression in C57BL/6 mice.

Materials and Methods

Mice. Five-6-week-old C57BL/6 male mice (AntiCancer, Inc., San Diego, CA, USA) were used in the present study. All experiments were performed according to the National Institutes of Health (NIH) Guide for the Care and Use of Animals, with assurance number A3873-1, as described previously (9-10).

Orthotopic-transplantation model of mouse colon-cancer cell line MC38. The mouse colon-cancer cell line MC38 was grown in high-glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 100 IU/ml penicillin/streptomycin. C57BL/6 male mice were initially injected subcutaneously with 10^6 MC38 cells in the right flank to make stock tumors for harvest and preparation of tumor fragments. Two-mm^3 fragments of MC38 were prepared. One tumor fragment was sutured to the surface of the cecum with a single stitch using 7-0 sutures (Ethicon, Inc., NJ, USA). The cecum was returned to the abdominal cavity. The wound was closed with 6-0 sutures (Ethicon, Inc., NJ, USA) (8).

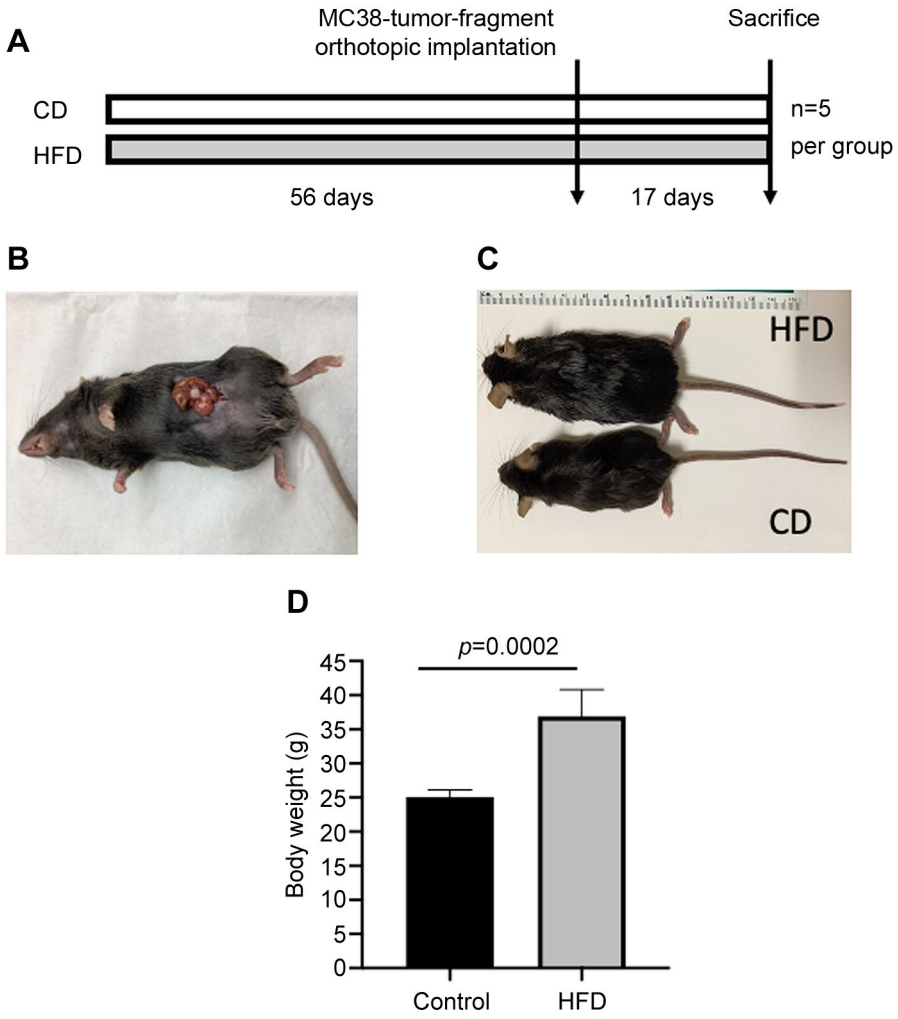

Figure 1. *(A) Experimental schema. (B) Growth of an orthotopic-transplantation model of MC38 colon cancer in C57BL/6 mice. (C) Mice on HFD or CD diets. (D) Body weight of mice on the day of orthotopic transplantation of MC38. Error bars: ± Standard error of the mean (SEM). HFD: High-fat diet; CD: control diet.*

High-fat diet induced-obesity. HFD-induced obesity was established in C57BL/6 mice as previously reported (9, 10). Briefly, 5-week-old male C57BL/6 mice were fed either a control diet (CD) (6.5% fat, Teklad 2020x, Harlan Laboratories, Indianapolis, IN, USA) or HFD (34.3% fat, Teklad TD.06414, Harlan Laboratories) for eight weeks (Figure 1A). After eight weeks on the HFD (n=5) or CD (n=5), each group was orthotopically implanted with an MC38 tumor fragment in the cecum (Figure 1B). Each group was maintained on the initial diet until the end of the experiment. All mice were sacrificed on day 17. The tumor size and tumor weight were measured at the time of sacrifice. Tumor volume was calculated with the following formula: tumor volume (mm^3)=length (mm) × width (mm) × width (mm) × 1/2.

Statistical analysis. All data are presented as mean±standard error of the mean (SEM). *p*-Values were calculated with the Student's unpaired t-test. A *p*-value ≤0.05 was considered significant. Statistical analyses were performed using GraphPad Prism 8.4.3 (GraphPad Software, Inc., San Diego, CA, USA).

Results

HFD-induced obesity model. The mean body weight of the mice on the HFD group (n=5) was 36.04±1.60g and the mean body weight of the mice on the CD (n=5) was 25.08±0.47g at the time of orthotopic transplantation of MC38 tumor fragments (*p*=0.0002) (Figure 1C and D).

Volume of MC38 tumors from C57BL/6 mice on HFD and CD. The mean volume of the MC38 tumors in the HFD group (n=5) was 1698.01±400.13 mm^3 and the mean tumor volume

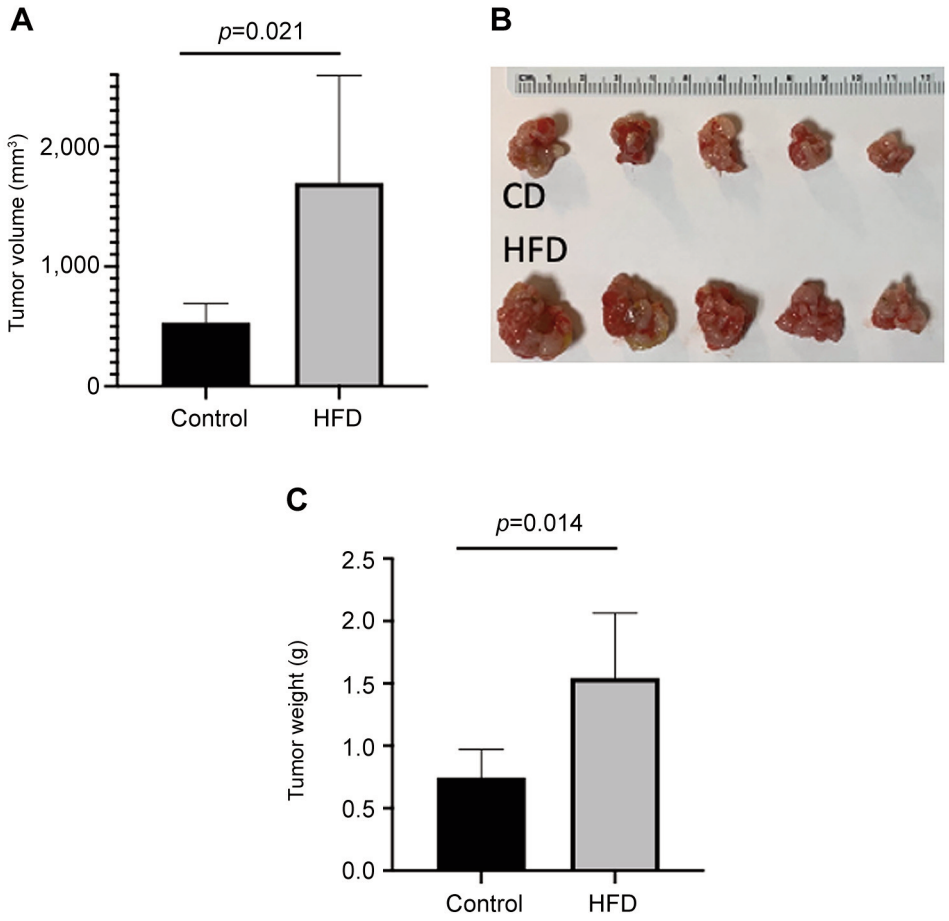

Figure 2. *Effect of an HFD on the growth of MC38 colon cancer in the syngeneic orthotopic model in C57BL/6 mice. (A) Tumor volume of MC38 colon cancer in mice fed with HFD or CD. (B) MC38 colon tumors excised from C57BL/6 mice on CD or HFD. (C) Weight of MC38 tumors from mice on HFD or CD. Error bars: ±Standard error of the mean (SEM). HFD: High-fat diet; CD: control diet.*

in the CD group (n=5) was 532±71 mm^3 (p=0.021) 17 days after orthotopic transplantation (Figure 2A and B).

Tumor weight of MC38 in HFD-induced obesity mice. The mean weight of the MC38 tumors in the HFD group (n=5) was 1.54±0.2 g and the tumor weight of the tumors in the CD group was (n=5) was 0.75±0.1g (p=0.014), 17 days after transplantation (Figure 2C).

Discussion

The HFD increased colon-cancer growth in an orthotopic transplantation model of MC38 in C57BL/6 mice more than twice compared to the CD in the present study. MC38 subcutaneous tumor growth in C57BL/6 mice was increased on a HFD (4, 5, 11). However, orthotopic models of cancer are more patient-like (6, 8, 12). It was reported that there were fewer $CD8^+$ T cells in tumors subcutaneously growing in HFD-induced obese mice, compared to subcutaneous tumors in mice on a CD (6), indicating that HFD-induced obesity may impair $CD8^+$ T-cell function (6). MC38 tumors are sensitive to immune checkpoint inhibitors (ICIs). However, the therapeutic efficacy of ICIs is limited. Obesity may be one of the reasons for its limited efficacy. In addition, long-chain fatty acids have been implicated in tumorigenesis (13). These parameters will be investigated in future studies in orthotopic syngeneic mouse-cancer models.

In a previous study of mouse CT26 colon cancer, orthotopically-implanted in BALB/c mice fed a HFD had an increased frequency of metastasis; however, primary tumor growth was not affected (14). The CT26 tumor was implanted using a glue, instead of sutures, as in the present study, which may have affected the response of the primary tumor to the HFD.

Future studies will investigate the role of a HFD on metastasis in the MC38 colon-cancer C57BL/6 syngeneic orthotopic model.

Conclusion

The results of the present study emphasize the strong effect of obesity on colon cancer progression.

Conflicts of Interest

K.H., Y.K., Y.A., N.S., J.Y., Y.T., and R.M.F. are unsalaried associates of AntiCancer Inc. The Authors declare no competing financial interests in relation to this study.

Authors' Contributions

K.H. and R.M.H designed and performed experiments, analyzed data, and wrote the article; Y.K., Y.A., J.Y., N.S., Y.T. provided technical support and conceptual advice.; K.H., T.T., and R.M.H. reviewed and revised the manuscript.

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