Increased dietary fatty acids determine the fatty-acid profiles of human pancreatic cancer cells and their carrier’s plasma, pancreas and liver

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Abstract. Primary contents of dietary fat are three or four types of fatty acids, namely saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), n6-polyunsaturated fatty acid (n6PUFA) and, to less extent, n3-polyunsaturated fatty acid (n3PUFA). Previous studies suggest that increased SFA, MUFA, and n6PUFA in high fat diets (HFDs) stimulate the origination, growth, and liver metastasis of pancreatic cancer cells, whereas increased n3PUFA has the opposite effects. It is unclear whether the fatty acid-induced effects are based on changed fatty-acid composition of involved cells. Here, we investigated whether increased SFA, MUFA, n6PUFA, and n3PUFA in different HFDs determine the FA profiles of pancreatic cancer cells and their carrier’s plasma, pancreas, and liver. We transplanted MiaPaCa2 human pancreatic cancer cells in athymic mice and fed them normal diet or four HFDs enriched with SFA, MUFA, n6PUFA, and n3PUFA, respectively. After 7 weeks, fatty acids were profiled in tumor, plasma, pancreas, and liver, using gas chromatography. When tumor carriers were fed four HFDs, the fatty acids that were increased dietarily were also increased in the plasma. When tumor carriers were fed MUFA-, n6PUFA-, and n3PUFA-enriched HFDs, the dietarily increased fatty acids were also increased in tumor, pancreas, and liver. When tumor-carriers were fed the SFA-enriched HFD featuring lauric and myristic acids (C12:0 and C14:0), tumor, pancreas, and liver showed an increase not in the same SFAs but palmitic acid (C16:0) and/or stearic acid (C18:0). In conclusion, predominant fatty acids in HFDs determine the fatty-acid profiles of pancreatic cancer cells and their murine carriers.

Key words: High fat diet, Fatty-acid profile, Pancreatic cancer cells, Pancreas, Liver

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HABITUAL INTAKE OF HIGH FAT DIET (HFD) induces obesity and insulin resistance and causes type-2 diabetes and blood-vascular disease [1]. The obesity and insulin resistance also increase cancer risk at organs such as pancreas, big intestine, and mammary glands [2]. Three types of fatty acids (FAs), namely saturated FA (SFA), monounsaturated FA (MUFA), and omega-6 polyunsaturated FA (n6PUFA), constitute the bulk of dietary fat [3]. Total dietary FAs may derive from these types largely equally or derive mainly from one or two of them [3]. As a 4th FA type, omega-3 polyunsaturated fatty acid (n3PUFA) is scarce in most food materials but rich in fish oil and flaxseed [3]. Epidemiologic studies have shown that diet-derived SFA and n6PUFA increase the risk of pancreatic cancer, whereas n3PUFA decreases the risk [4, 5]. As for dietary MUFA, however, its association with pancreatic-cancer risk was either positive or inverse in different studies [4, 6]. When rodents were treated with pancreatic carcinogens or were genetically prone to pancreatic cancer, the genesis and growth of pancreatic cancer cells were stimulated by SFA-, MUFA-, and n6PUFA-enriched diets and inhibited by n3PUFA-enriched diet [7-14]. When mature human pancreatic cancer cells were transplanted in the pancreas of athymic mice, they were stimulated by SFA-, MUFA-, and n6PUFA-enriched diets and inhibited
by n3PUFA-enriched diet [15, 16]. In vitro, the growth of pancreatic cancer cells was increased by SFA and MUFA, inhibited by n3PUFA, and diversified in the presence of n6PUFA [16-19].

Dietary FAs may also regulate liver metastasis of pancreatic cancer cells [16, 20, 21]. When we transplanted pancreatic cancer cells in the pancreas of athymic mice, liver metastasis was stimulated by dietarily increased n6PUFA and inhibited by n3PUFA [16]. In the same study, the HFDs that were enriched with n6PUFA or n3PUFA also increased the same PUFA in the liver, which suggests that diet-induced changes in hepatic FA composition may precede diet-induced liver metastasis. In that study, however, the liver examined may contain metastatic tumors [16]. Thus, whether hepatic FA composition influences pancreatic-cancer metastasis is uncertain. Similarly uncertain is whether dietary FAs determine the FA profiles of normal and cancerous pancreases.

We undertook the present study to investigate whether increased SFA, MUFA, n6PUFA, and n3PUFA in different HFDs determine the FA profiles of pancreatic cancer cells and their carrier’s plasma, pancreas, and liver. To that end, we made four HFDs featuring the four FA types and gave them to the athymic mice that carried MiaPaCa2 human pancreatic cancer cells. Tumor-free and tumor-carrying athymic mice living on normal mouse chow were used as references. After 7 weeks, FAs were profiled in tumor, plasma, pancreas, and liver, using gas chromatography. MiaPaCa2 cells were transplanted subcutaneously, rather than orthotopically, in order to keep pancreas and liver from local and metastatic cancer cells for the sake of FA profiling.

Materials and Methods

Mice and diets

The ethics committee of Tianjin Hospital of Integrated Traditional Chinese and Western Medicine approved this study (Approval No. NKYY-DWLL-2019-033). Male athymic Balb/c mice were bought from Hua-Fu-Kang Bioscience (Beijing, China). When animal arrived, they were 4–5 weeks old and weighed ~20 g. In our institution, they lived in a room with 12 h/12 h light/dark cycle and had free access to food and water. During acclimation, mice were fed a normal mouse diet produced by Hua-Rong Animal Science and Technology (Tianjin, China). The normal diet (ND) was composed of flour (62%), soybean (24%), raw protein (8%), minerals, and vitamins and had 5% (w/w) fat mainly from soybean. We purchased cocoanut, olive, flaxseed, and soybean oils from a local market. Wilmar Food Inc. (Tianjin, China) produced the flaxseed oil (GBT8235). Team Asia Corporation in Singapore produced the cocoanut oil.

ND was ground, and the powder was mixed with the four oils, respectively, giving four HFDs. The additional oil in each HFD accounted for 15% of the final weight. Given that ND had 5% (w/w) fat, the HFDs had 20% (w/w) fat unanimously and were enriched with SFA, MUFA, n6PUFA, and n3PUFA, respectively. We also performed a prior experiment to double-check the FA composition of the oils, using gas chromatography.

Mouse management

MiaPaCa2 human pancreatic cancer cells (#CC2408) were purchased from the Cell Bank of Chinese Academy of Science (Shanghai, China) and cultured in serum-containing media. Three millions of MiaPaCa2 cells were transplanted subcutaneously in an athymic mouse. After a tumor formed (φ = 1 cm), it was harvested and cut into small pieces (1–2 mm³). A piece of tumor was transplanted subcutaneously in each designated mouse as we described previously [22]. One week later, all tumor carriers were divided into 5 groups (8–9 mice per group). From then on, four groups of tumor carriers were fed four HFDs, respectively, and the last group still fed ND. A group of intact athymic mice (n = 5) were fed ND as well. After 7 weeks, all mice were sacrificed under anesthesia [22]. Blood was collected from the orbital sinus, and plasma separated. The pancreases, liver, and subcutaneous tumor were resected [22].

Gas chromatography

Before a piece of tissue sample (50 μg) was processed, methylbenzene (1 mL) and petroleum ether (1 mL) were mixed. The tissue was transferred to the buffer and homogenized with a mechanical homogenizer. When plasma or plant oils were processed, the sample (200 μL) was added to the methylbenzene/petroleum-ether mix directly. The preparation of tissue or plasma was centrifuged (3,000 × g, 10 min), and the upper layer was transferred to 2 mL of methanol with 5% NaOH. It was incubated at 30°C for 30 min and then mixed with the same volume of water. The mixture was rested at room temperature for 1 h, and the upper layer was collected and evaporated with helium. After being suspended in dichloromethane (0.5 mL), the preparation was filtered (pore size: 0.45 μm). n-Pentadecane (Sigma, 442700) was added in the concentration of 0.5 mg/mL. A 37 fatty-acid mix was bought from Sigma (Supelco 37 component FAME mix) and diluted with pentadecane-containing dichloromethane.

Shimadzu-GC-2014 gas chromatographer was equipped with a flame ionization detector, an SP2560 Capillary GC Column (24056, Sigma-Aldrich), and the software of
Lab Solutions (Version 5.54 SP5, 2011 Shimadzu Corporation). Sample preparation was injected in the volume of 1 μL. Injector and detector temperatures were 250 and 260°C, respectively. Helium was used as carrier gas, and the split ratio was 50:1. Oven temperature started from 100°C (5 min) and increased at a rate of 4°C per minute. When temperature reached 240°C, it was maintained for 30 min. Any FA was identified by retention time and then quantified by measuring spike area. After all FAs were quantified in a sample, their results were aggregated to give the total concentration. Using the total concentration as 100%, the contribution (%) of each FA was calculated.

Statistics Data shown are means ± SEM. We used the analysis of variance to assess difference in groups and used least significant difference for post-hoc test. The software of Statistical Product and Service Solutions (version 17.0) was used. P < 0.05 was considered significant.

Results

The FA composition of used oils
When we examined the FA composition of four oils, the contribution by each FA was shown in the percent of oil weight, as in Reference 3. As a result, we found all the FAs that were listed in that reference and also found a total of five FAs that were not listed therein (Table 1). As expected, the cocoanut oil was enriched with saturated lauric and myristic acids; the olive oil enriched with monounsaturated oleic acid; the soybean oil enriched with n6-polyunsaturated linoleic acid, and the flaxseed oil enriched with n3-polyunsaturated linolenic acid (Table 1).

Animal survival, food intake, and body weight
All tumor-free mice survived the experiment, and so did the tumor carriers living on ND. Two or three mice were removed from each group of HFD-fed tumor carriers, because the mice either had poor condition due to tumor burden or lacked tumor. Similar food intake and body weight gain were seen in the two groups of ND-fed mice that were either tumor-free or tumor carrying (Table 2). The tumor carriers living on MUFA- and n3PUFA-enriched HFDs had greater food intake than those living on ND. The tumor carriers living on n6PUFA-enriched HFD had less body-weight gain than ND-fed tumor carriers (Table 2).

Plasma FAs
A total of 12 fatty acids were found in plasma samples (Table 3). When we assessed FA profiles, we regarded the FAs that contributed more than 10% of total FAs as major contributors. In intact athymic mice, palmitic acid (C16:0), stearic acid (C18:0), and linoleic acid (C18:2, n6) were such contributors to total FAs in the plasma. When plasma FA profiles were compared between the ND-fed mice that were tumor-free or tumor carrying, no significant differences were seen (Table 3).

In the plasma of the tumor carriers living on four HFDs, palmitic, stearic, and linoleic acids remained major FAs (Table 3). In addition, each HFD impacted plasma FA profile in its own way: When mice were fed the HFD enriched with lauric and myristic acids, the same SFAs were increased in the plasma (Table 3). When mice were fed oleic acid-enriched HFD, this

| fatty acids          | cocoanut oil | olive oil | soybean oil | flaxseed oil |
|----------------------|--------------|-----------|-------------|--------------|
| caprylic acid (C8:0) | 3.39 ± 1.70  | —         | —           | —            |
| capric acid (C10:0)  | 3.70 ± 1.29  | —         | —           | —            |
| lauric acid (C12:0)  | 57.96 ± 3.77 | —         | —           | —            |
| myristic acid (C14:0)| 23.93 ± 2.09 | —         | —           | 0.02 ± 0.01  |
| palmitic acid (C16:0)| 3.99 ± 0.97  | 8.12 ± 2.47 | 7.02 ± 1.63 | 4.96 ± 0.74  |
| stearic acid (C18:0) | 1.66 ± 0.41  | 2.71 ± 0.81 | 3.10 ± 0.79 | 3.45 ± 0.41  |
| palmitoleic acid (C16:1)| —         | 0.61 ± 0.18 | 0.02 ± 0.01 | 0.02 ± 0.01  |
| oleic acid (C18:1)   | 3.51 ± 0.66  | 77.20 ± 6.62 | 26.76 ± 5.79 | 22.16 ± 2.69 |
| linoleic acid (C18:2, n6)| 1.23 ± 0.41 | 8.50 ± 2.71 | 53.58 ± 4.87 | 20.41 ± 2.08 |
| eicosadienoic acid (C20:2, n6)| —         | —         | 0.05 ± 0.04 | —            |
| icosatrienioic acid (C20:3, n6)| —         | 0.40 ± 0.27 | —           | —            |
| linolenic acid (C18:3, n3)| —         | 1.56 ± 0.61 | 8.97 ± 4.40 | 40.43 ± 4.71 |

Data are from three tests. Data in parentheses are control values coming from a previous report (Herausgegeben 1994).
MUFA became the most populous FA in the plasma (Table 3). Although linoleic acid already was a major FA in the plasma of ND-fed mice, plasma linoleic acid was further increased when mice were fed the HFD enriched with this particular n6PUFA (Table 3). Linolenic acid was not found in the plasma of the mice fed ND. When this n3PUFA was enriched in diet, however, it was found in the plasma and accounted for 10% of total FAs therein (Table 3).

**Tumorous FAs**

When tumor carriers were fed ND, palmitic, stearic, oleic, and linoleic acids were major contributors to total tumorous FAs (Table 4). When tumor carriers were fed the HFD enriched with lauric and myristic acids (C12:0 and C14:0), tumor grafts showed an increase not in the same SFAs but two longer ones, namely palmitic and stearic acids (C16:0 and C18:0, Table 4). When tumor-carrying mice were fed oleic acid-enriched HFD, the same MUFA accounted for 53% of tumorous FAs (Table 4). When tumor carriers were fed linoleic acid- or linolenic acid-enriched HFDs, the same PUFAs were increased in tumor grafts (Table 4).

**Pancreatic FAs**

When tumor-free and tumor-carrying mice were fed ND, myristic, palmitic, stearic, linoleic, and arachidonic acids were major FAs in the pancreas (Table 5). When tumor-carrying mice were fed the HFD enriched with lauric and myristic acids, the pancreas showed an increased not in the same SFAs but in palmitic acid (Table 5). When tumor-carrying mice were fed oleic acid-enriched HFD, this MUFA accounted for 59% of pancreatic FAs (Table 5). When tumor carriers were fed linoleic acid- or linolenic acid-enriched HFD, the same PUFA was increased in the pancreas (Table 5).

Tentatively, we examined tumorous vs. pancreatic FA profiles in the same mice (Tables 4 and 5). When tumor carriers were fed ND, palmitic, stearic, oleic, and linoleic acids were major contributors to total tumorous FAs (Table 4). When tumor carriers were fed the HFD enriched with lauric and myristic acids (C12:0 and C14:0), tumor grafts showed an increase not in the same SFAs but two longer ones, namely palmitic and stearic acids (C16:0 and C18:0, Table 4). When tumor-carrying mice were fed oleic acid-enriched HFD, the same MUFA accounted for 53% of tumorous FAs (Table 4). When tumor carriers were fed linoleic acid- or linolenic acid-enriched HFDs, the same PUFAs were increased in tumor grafts (Table 4).

**Table 2** Food intake and 8-week body weight gain in tumor-free or tumor-carrying mice fed normal diet or one of 4 high fat diets

|                      | tumor-free (g/day) | tumor-carrying (g/day) |
|----------------------|--------------------|------------------------|
| food intake          | 4.77 ± 0.12 (5)    | 4.70 ± 0.10 (9)        |
| body weight gain     | 4.56 ± 0.63 (5)    | 5.08 ± 0.27 (9)        |

* p < 0.05 and ** p < 0.01 between the indicated tumor-carrying mice and those that were fed normal diet. Animal numbers are shown in the parentheses.

**Table 3** Fatty acids in the plasma of tumor-free and tumor-carrying mice fed normal diet or one of 4 high fat diets

| fatty acids                     | tumor-free (g/day) | tumor-carrying (g/day) |
|--------------------------------|--------------------|------------------------|
| undecanoic acid (C11:0)        | 0.29 ± 0.29        | 0.16 ± 0.16            |
| lauric acid (C12:0)            | 35.21 ± 2.65       | 30.10 ± 3.94           |
| oleic acid (C18:1)             | 4.07 ± 2.14        | 4.10 ± 1.28            |
| linoleic acid (C18:2, n6)      | 4.46 ± 1.89        | 4.77 ± 0.81            |
| α-linolenic acid (C18:3, n3)   | —                  | 4.77 ± 0.81            |
| eicosapentaenoic acid (C20:5, n3) | —        | 0.60 ± 0.60            |
| docosahexaenoic acid (C22:6, n3) | —                  | 0.75 ± 0.75            |

* p < 0.05 and ** p < 0.01 between the indicated tumor carriers and those fed any other diets. p < 0.01 between the indicated tumor carriers and those fed normal diet or fed n6PUFA and n3PUFA-enriched high fat diets.
Table 4  Fatty-acid profiles in 5 groups of tumor grafts whose hosts were fed normal diet or one of 4 high fat diets

| fatty acids | normal diet | high fat diets featuring different types of fatty acids |
|-------------|-------------|--------------------------------------------------------|
|             | normal diet | SFA  | MUFA | n6PUFA | n3PUFA |
|             | n = 9 | n = 6 | n = 6 | n = 7 | n = 6 |
| undecanoic acid (C11:0) | 0.20 ± 0.10 | 0.45 ± 0.30 | 0.09 ± 0.06 | 0.02 ± 0.02 | 0.10 ± 0.10 |
| lauric acid (C12:0) | — | — | — | — | 0.49 ± 0.29 |
| tridecanoic acid (C13:0) | 0.15 ± 0.09 | 0.76 ± 0.32 | 0.09 ± 0.06 | 1.00 ± 0.48 | 0.31 ± 0.14 |
| myristic acid (C14:0) | 2.65 ± 1.57 | 4.42 ± 2.31 | 2.00 ± 1.22 | 2.44 ± 1.00 | 6.92 ± 3.70 |
| palmitic acid (C16:0) | 26.74 ± 1.49 | 33.95 ± 2.68* | 19.20 ± 2.52 | 23.53 ± 1.75 | 20.95 ± 2.49 |
| stearic acid (C18:0) | 11.87 ± 2.05 | 24.10 ± 2.44** | 10.42 ± 2.43 | 13.23 ± 1.93 | 13.04 ± 3.42 |
| myristoleic acid (C14:1) | 0.09 ± 0.08 | — | — | — | — |
| palmitoleic acid (C16:1) | 5.93 ± 0.73 | 0.62 ± 0.42 | 2.07 ± 0.44 | 1.57 ± 0.59 | 3.47 ± 0.65 |
| oleic acid (C18:1) | 22.11 ± 1.50 | 14.96 ± 2.34 | 52.76 ± 4.51** | 20.87 ± 1.35 | 24.40 ± 4.12 |
| linoleic acid (C18:2, n6) | 29.62 ± 1.93 | 17.01 ± 3.25 | 11.64 ± 2.52 | 36.45 ± 2.54* | 21.76 ± 2.41 |
| γ-linolenic acid (C18:3, n6) | 0.07 ± 0.04 | — | 0.12 ± 0.07 | 0.08 ± 0.07 | 0.81 ± 0.21 |
| eicosadienoic acid (C20:2, n6) | — | — | — | 0.03 ± 0.03 | — |
| arachidonic acid (C20:4, n6) | 0.25 ± 0.11 | 0.12 ± 0.12 | 0.40 ± 0.28 | — | — |
| α-linolenic acid (C18:3, n3) | 0.33 ± 0.11 | — | — | 0.54 ± 0.23 | 7.75 ± 1.85** |

* p < 0.05 and ** p < 0.01 between the indicated group and any other groups.

Table 5  Profiles of fatty acids in the pancreas of tumor-free and tumor-carrying mice fed normal diet or one of 4 high fat diets

| fatty acids | mice fed normal diet | tumor carriers fed 4 high fat diets featuring different types of fatty acids |
|-------------|----------------------|-----------------------------------------------------------------------------|
|             | tumor-free | tumor-carrying | SFA  | MUFA | n6PUFA | n3PUFA |
|             | n = 5 | n = 9 | n = 6 | n = 6 | n = 7 | n = 6 |
| caprylic acid (C8:0) | 1.95 ± 1.25 | 0.12 ± 0.12 | — | 0.40 ± 0.28 | — | — |
| lauric acid (C12:0) | 2.10 ± 0.58 | 1.81 ± 0.44 | 1.17 ± 0.83 | 0.16 ± 0.10 | 0.18 ± 0.18 | 0.21 ± 0.21 |
| tridecanoic acid (C13:0) | 6.90 ± 1.57 | 2.72 ± 0.47 | 2.08 ± 0.46 | 1.16 ± 0.66 | 1.84 ± 0.44 | 1.75 ± 0.49 |
| myristic acid (C14:0) | 19.64 ± 3.80 | 23.14 ± 3.46 | 14.01 ± 3.26 | 3.86 ± 1.80 | 7.13 ± 1.20 | 10.39 ± 2.81 |
| palmitic acid (C16:0) | 15.67 ± 1.83 | 21.02 ± 0.94 | 26.56 ± 1.09** | 15.05 ± 0.82 | 17.87 ± 0.84 | 20.56 ± 0.95 |
| stearic acid (C18:0) | 11.07 ± 0.85 | 10.28 ± 0.88 | 9.94 ± 1.45 | 4.97 ± 1.27 | 9.29 ± 1.08 | 9.75 ± 1.64 |
| myristoleic acid (C14:1) | 3.71 ± 2.43 | 8.22 ± 1.72 | 3.00 ± 1.04 | 0.72 ± 0.70 | 4.08 ± 1.09 | 4.06 ± 1.28 |
| palmitoleic acid (C16:1) | 1.09 ± 0.72 | 0.59 ± 0.47 | 2.15 ± 0.85 | 1.61 ± 0.61 | 0.21 ± 0.12 | 1.39 ± 0.91 |
| trans-9-elaidic acid (C18:1) | 2.24 ± 0.79 | 0.09 ± 0.09 | — | 0.05 ± 0.04 | — | — |
| oleic acid (C18:1) | 9.41 ± 4.59 | 7.68 ± 3.56 | 19.94 ± 5.13 | 59.36 ± 5.75** | 14.04 ± 3.90 | 22.72 ± 4.07 |
| cis-11-eicosenoic acid (C20:1) | — | 0.07 ± 0.07 | 0.09 ± 0.06 | 0.47 ± 0.16 | 0.13 ± 0.07 | 0.23 ± 0.15 |
| linoleic acid (C18:2, n6) | 15.86 ± 1.52 | 12.00 ± 1.02 | 14.48 ± 0.81 | 10.34 ± 0.72 | 37.70 ± 0.94** | 18.04 ± 0.62 |
| arachidonic acid (C20:4, n6) | 10.36 ± 0.99 | 12.25 ± 0.56 | 6.39 ± 1.37 | 1.80 ± 1.13 | 6.71 ± 1.38 | 0.05 ± 0.05 |
| α-linolenic acid (C18:3, n3) | — | — | 0.02 ± 0.02 | 0.06 ± 0.04 | 0.46 ± 0.24 | 10.60 ± 0.43** |
| docosahexaenoic acid (C22:6, n3) | — | — | 0.16 ± 0.12 | — | 0.36 ± 0.36 | 0.25 ± 0.25 |

* p < 0.05 and ** p < 0.01 between the indicated tumor carriers and any other tumor carriers.
When tumor-free and tumor-carrying mice lived on ND, the FA profile of MiaPaCa2 cell-made tumor was characterized by an increase in oleic acid (C18:1) in the pancreas; 22.11% vs. 7.68% and linoleic acid (C18:2 n6) in tumor vs. pancreas: 29.62% vs. 15.86%. While SFA-enriched HFD only increased palmitic acid (C16:0) in the pancreas, it increased both palmitic acid and stearic acid (C18:0) in the tumor.

**Hepatic FAs**

When tumor-free and tumor-carrying mice lived on ND, myristic, palmitic, stearic, oleic, and linoleic acids were major FAs in the liver (Table 6). When tumor-carrying mice were fed the HFD enriched with lauric and myristic acids, their liver showed an increase not in the same SFAs but in palmitic acid (C16:0, tumor-carrying mice). When tumor-carrying mice were fed MUFA- and n6PUFA-enriched HFDs, the dietarily increased FAs were also increased in the liver (Table 6). When tumor carriers were fed the HFD enriched with n3-polyunsaturated linolenic acid, the same n3PUFA and a longer one, namely eicosapentaenoic acid (C22:6), were both increased in the liver (Table 6).

### Table 6  Fatty acids in the liver of tumor-free and tumor-carrying mice that were fed normal diet or one of 4 high fat diets

| fatty acids             | mice fed normal diet | tumor carriers fed 4 high fat diets featuring different types of fatty acids |
|-------------------------|----------------------|--------------------------------------------------------------------------------|
|                         | tumor-free           | tumor-carrying                                                               | SFA                              |
|                         | $n = 5$              | $n = 9$                                                                      | $n = 6$                          |
| undecanoic acid (C11:0) | 1.14 ± 0.64          | 2.30 ± 0.37                                                                  | 4.48 ± 2.51                      |
| tridecanoic acid (C13:0)| 1.32 ± 0.55          | 1.69 ± 0.23                                                                  | 2.61 ± 0.88                      |
| myristic acid (C14:0)   | 16.39 ± 4.47         | 13.78 ± 2.38                                                                 | 12.04 ± 2.06                     |
| palmitic acid (C16:0)   | 18.19 ± 1.74         | 20.37 ± 1.11                                                                 | 22.67 ± 1.65a                    |
| stearic acid (C18:0)    | 12.06 ± 2.18         | 11.67 ± 0.58                                                                 | 9.32 ± 0.84                      |
| tricosanoic acid (C23:0)| 0.03 ± 0.03          | 1.97 ± 0.54                                                                  | 0.29 ± 0.29                      |
| myristoleic acid (C14:1)| 1.67 ± 0.86          | 1.29 ± 0.56                                                                  | 0.56 ± 0.40                      |
| palmitoleic acid (C16:1)| 1.16 ± 1.06          | 0.41 ± 0.27                                                                  | 0.47 ± 0.47                      |
| oleic acid (C18:1)      | 17.98 ± 4.21         | 14.22 ± 2.50                                                                 | 18.65 ± 3.42                     |
| linolelaidic acid (C18:2, n6)| 6.94 ± 3.02 | 10.89 ± 3.34                                                                 | 11.80 ± 3.48                     |
| linoleic acid (C18:2, n6)| 13.88 ± 2.78         | 13.40 ± 1.69                                                                 | 10.20 ± 0.99                     |
| gamma-linolenic acid (C18:3, n6)| 0.13 ± 0.13 | 0.14 ± 0.14                                                                  | —                               |
| arachidonic acid (C20:4, n6)| 7.39 ± 1.73 | 6.20 ± 0.91                                                                  | 6.30 ± 1.17                      |
| $\alpha$-linolenic acid (C18:3, n3)| 1.73 ± 0.93 | 0.07 ± 0.07                                                                  | 0.62 ± 0.62                      |
| eicosapentaenoic acid (C20:5, n3)| — | —                                                                           | 0.48 ± 0.31                      |
| docosahexaenoic acid (C22:6, n3)| — | 1.59 ± 0.70                                                                  | 1.38 ± 0.63                      |

**p < 0.01 between indicated tumor carriers and any other tumor carriers. *p < 0.01 between the indicated tumor carriers and those fed other high fat diets.**

**Discussion**

In 2010, Gong and co-workers demonstrated that the risk of pancreatic cancer was both positively associated with eight dietary SFAs, two MUFAs (palmitoleic and oleic acids) and an n6PUFA (linoleic acid) and inversely associated with n3PUFAs [4]. In that context, we previously made SFA-, MUFA-, n6PUFA-, and n3PUFA-enriched HFDs from scratch and gave them to different athymic mice that carried HPAF-2 pancreatic cancer cells [16]. Whilst the SFA-, MUFA-, and n6PUFA-enriched HFDs stimulated the cancer, the 3PUFA-enriched HFD inhibited the cancer [16]. Interestingly, the use of the four HFDs decreased both food intake and body-weight gain for unknown reasons [16]. In the present study, we made four HFDs using ND as a basic food. The mice ate more MUFA- or n3PUFA-enriched HFDs than ND, whereas the tumor carriers living on n6PUFA-enriched HFD had less body-weight gain than those living on ND.

Previous studies have shown pancreatic cancer is associated with specific plasma FA profiles [23, 24]. In the
present study, however, plasma FA profiles were similar in the ND-fed mice that carried tumor or not. The lack of cancer-specific FA profile may be related to the individuality of MiaPaCa2 cells and to the subcutaneous location of the tumor. When tumor-carrying athymic mice were fed different HFDs, the FAs that were increased in the HFDs were also increased in the plasma. Further, the MUFA-, n6PUFA-, and n3PUFA-enriched HFDs also increased the corresponding FAs in tumor, pancreas, and liver. When mice were fed the HFD enriched with lauric and myristic acids (C12:0 and C14:0), tumor grafts, pancreas, and liver showed an increase not in the same SFAs but in a longer one, i.e., palmitic acid (C16:0). This suggests that the diet-derived SFAs were elongated after they entered cells. This notion is consistent with the knowledge that palmitic acid plays an important role in fatty-acid synthesis [25].

The tumors whose carriers were fed the SFA-enriched HFD also showed an increase in an even longer SFA, namely stearic acid (C18:0). Because this SFA may be elongated from palmitic acid, the present result was in line with the knowledge that FA elongation is increased in cancer cells [26]. In addition, this result is also in keeping with the notion that increased stearic acid is a characteristic of the FA profiles in cancer cells [27]. Studies have shown that stearic acid is converted to oleic acid by virtue of stearol-CoA desaturase, and the latter fatty acid is in turn involved in the biology of cancer cells [28, 29].

When we compared the extents to which different HFDs increased corresponding FAs in both tumor and mice, we found that the oleic acid-enriched HFD was the most efficient in that regard. For instance, when tumor carriers were fed the given HFD, oleic acid was increased to >50% of total FAs in both tumor graft and pancreas. When mice were fed ND, oleic acid contributed only 3–4% of total plasma FAs in both tumor-free and tumor-carrying conditions. When tumor-carrying mice were fed oleic acid-enriched HFD, however, this MUFA became the most populous FA in the plasma. All these results suggest that changing the amount of dietary oleic acid may effectively change the magnitude of oleic-acid-induced effects on pancreatic cancer cells. Experimental evidence has suggested that oleic acid stimulates pancreatic cancer cells [9, 16, 17]. However, when dietary oleic acid was related to the risk of pancreatic cancer in epidemiologic studies, both positive and inverse associations were found [4, 6]. More studies are required to clarify why these epidemiologic results were not consistent.

When fat emulsions that featured MUFA, n6PUFA and n3PUFA, respectively, were given to piglets intravenously, the same types of FAs were increased in the pancreas [30]. When weaning rats were fed MUFA- and n6PUFA-enriched HFDs, the same FAs were increased in the pancreas [31]. Similar to these results, we demonstrated that, when unsaturated FAs were increased in diet, the same FAs were increased in the pancreas. These results, together with FA-induced effects on pancreatic cancer, suggest that changes in pancreatic FA profile may be a prerequisite for dietarily increased FAs to regulate pancreatic cancer cells. In the present study, the subcutaneous tumor made of MiaPaCa2 human pancreatic cancer cells appeared to have more oleic acid (C18:1) and linoleic acid (C18:2, n6) than normal mouse pancreas. In addition, SFA-enriched HFD increased stearic acid (C18:0) in pancreatic cancer cells but not normal cells. In a previous study, FAs were profiled in human urothelial carcinoma, using adjacent normal tissue as reference [27]. As a result, the tumorous FA profile was characterized by increased stearic acid (C18:0) and oleic acid (C18:1).

The liver is the first organ for blood-borne pancreatic cancer cells to reach. When we transplanted HPAF-2 human pancreatic cancer cells in the pancreas of athymic mice, we found that liver metastasis was increased by n6PUFA-enriched diet and decreased by n3PUFA-enriched diet [16]. In addition, dietary FAs also regulated liver metastasis of colorectal cancer [32, 33]. In the present study, diet-derived unsaturated FAs induced an increase of the same FAs in the liver. These data suggest that the deposition of diet-derived FAs in liver may be a prerequisite for the FAs to regulate liver metastasis in pancreatic cancer.

In summary, dietary increase of SFAs, MUFAs, n6PUFAs, and n3PUFAs increased the same FAs in the plasma of the athymic mice that carried human pancreatic cancer cells. In addition, the dietary increase of MUFAs, n6PUFAs, and n3PUFAs also increased the same FAs in pancreatic cancer cells and in tumor carrier’s pancreas and liver. These changes may be a prerequisite for diet-derived FAs to regulate genesis, growth, and liver metastasis of pancreatic cancer. Although the HFD enriched with saturated lauric and myristic acids did not increase the same SFAs in tumor cells, pancreas and liver, it increased longer SFA(s) in these places. In conclusion, the types of predominant fatty acids in high fat diets determine the fatty-acid profiles of human pancreatic cancer cells and their murine carrier’s plasma, pancreas, and liver. Of note, the conclusion is based on evidence that was derived from an animal experiment with the present design. Whether the present results mimic the situation in pancreatic cancer patients deserves more investigation.
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Disclosure

None of the authors have any potential conflicts of interest associated with this research.

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