Adipocytes promote malignant growth of breast tumours with monocarboxylate transporter 2 expression via β-hydroxybutyrate

Chun-Kai Huang, Po-Hao Chang, Wen-Hung Kuo, Chi-Long Chen, Yung-Ming Jeng, King-Jen Chang, Jin-Yuh Shew, Chun-Mei Hu & Wen-Hwa Lee

Adipocytes are the most abundant stromal partners in breast tissue. However, the crosstalk between breast cancer cells and adipocytes has been given less attention compared to cancer-associated fibroblasts. Here we find, through systematic screening, that primary mammary gland-derived adipocytes (MGDAs) promote growth of breast cancer cells that express monocarboxylate transporter 2 (MCT2) both in vitro and in vivo. We show that β-hydroxybutyrate is secreted by MGDAs and is required to enhance breast cancer cells malignancy in vitro. Consistently, β-hydroxybutyrate is sufficient to promote tumorigenesis of a mouse xenograft model of MCT2-expressing breast cancer cells. Mechanistically we observe that upon co-culturing with MGDAs or treatment with β-hydroxybutyrate, breast cancer cells expressing MCT2 increase the global histone H3K9 acetylation and upregulate several tumour-promoting genes. These results suggest that adipocytes promote malignancy of MCT2-expressing breast cancer via β-hydroxybutyrate potentially by inducing the epigenetic upregulation of tumour-promoting genes.
Emerging evidence indicates that the tumour microenvironment plays a vital role in the initiation and progression of many cancers. In breast cancer, cancer-associated fibroblasts and tumour-associated macrophages promote breast cancer progression and metastasis by secreting growth factors and chemokines. Deciphering the underlying molecular mechanisms of crosstalk among heterotypic cells is of great interest because new targets for improving diagnosis or novel therapeutic strategies may be revealed.

Adipocytes are abundant stromal cells in human breast tissue. During breast cancer development, invasion of tumour cells through the basement membrane results in a close interaction between cancer cells and adipocytes. Several clinical observations demonstrate that local invasion of the adipose tissue correlates with poor prognosis, highlighting the importance of adipocytes in breast tumour progression. In addition to their function in energy metabolism and regulation of energy homeostasis, adipocytes also secrete hormone and various cytokines (also known as adipokines), which participate in cancer progression. For instance, IGF-1, leptin and interleukin-6 (IL-6) secreted from adipocytes promote breast cancer growth and invasion. The expression of metalloproteinase (MMP)-11/stromelysin-3 in adipocytes is induced by breast cancer cells at the tumour invasive front, indicative of a role in extracellular matrix remodelling during breast cancer development. Furthermore, it has been shown that adipocytes co-cultured with breast cancer cells exhibit decreased adiponectin concomitant with IL-6 overexpression. These studies suggest there is an intimate crosstalk between adipocytes and breast cancer cells. However, the underlying mechanism of how breast cancer cells interact with mature adipocytes is not fully understood.

In this communication, we provide a systematic investigation exploring this issue for breast cancer. We establish a co-culture system using breast cancer cells and adipocytes isolated from breast cancer mastectomy specimens (called mammary gland-derived adipocytes, MGDAs) to identify novel receptor(s) involved in the crosstalk between breast cancer cells and adipocytes. Through microarray analyses and RNAi knockdown screening, monocarboxylate transporter 2 (MCT2) is identified as a new player in MGDAs-mediated promotion of tumorigenic activity. Furthermore, either MGDAs co-culture or treatment of β-hydroxybutyrate, an intrinsic histone deacetylase (HDAC) inhibitor, increases histone H3K9 acetylation and induces expressions of IL-1β and LCN2 to enhance tumorigenicity in MCT2-expressing breast cancer cells. Consistently, elevated expressions of MCT2 as well as β-hydroxybutyrate-induced genes, IL-1β and LCN2, are significantly (P values: MCT2/IL-1β = 0.029; MCT2/LCN2 = 0.017, log-rank test) correlated with poor prognosis in breast cancer patients in two independent cohorts. These findings provide new insights into the connection between adipocytes and breast cancer malignancy.

Results

MGDAs promote growth of distinct subtypes of breast cancer.

To address whether MGDAs play a role in promoting breast tumour malignancy, a panel of estrogen receptor (ER)-positive (MCF7 and MDA-MB-361) and ER-negative (MDA-MB-231, MDA-MB-157, MDA-MB-468 and SK-BR3) breast cancer cell lines were used for soft agar colony formation assays with MGDAs co-cultured in the bottom layer. In this co-culture system, MGDAs and breast cancer cells are separated, but crosstalk can occur through diffusible factors. Human primary MGDAs were isolated from breast cancer specimens after mastectomy according to published protocols. As shown in Fig. 1A,B, MGDAs promoted colony formation of MCF7, MDA-MB-361, MDA-MB-231 and MDA-MB-157 cells, but not MDA-MB-468 and SK-BR3 cells. No strict correlation with the ERs status of the breast cancer cell lines was noted. Similar results were also obtained using two independent batches of MGDAs isolated from different breast cancer patients, suggesting that this MGDAs activity is not patient-specific. The colony number increased when greater numbers of MGDAs were used in the co-culture system (Supplementary Fig. 1C). Next, we examined the tumour formation ability of breast cancer cells co-cultured with MGDAs in a mouse xenograft tumour model. MGDAs and breast cancer cells were co-implanted subcutaneously instead of in the fat pad to minimize potential interference by mouse adipocytes. As shown in Fig. 1b,c, the MCF7, MDA-MB-361, MDA-MB-231 and MDA-MB-157 cells mixed with MGDAs grew faster and had greater tumour weights than the control groups. However, little or no difference was observed with the MDA-MB-468 cells (Fig. 1d). Taken together, these data suggest that promotion of breast cancer malignancy by MGDAs is selectively dependent on breast cancer cell type, with the effects likely mediated by soluble factors secreted from the MGDAs.

Identifying membrane proteins mediating responses to MGDAs.

To explore potential factors of breast cancer cells involved in MGDAs-mediated tumorigenic enhancement, we compared the gene expression profiles of MDA-MB-231, MDA-MB-361, MDA-MB-157 and MDA-MB-468 cells from previous cDNA microarray results. As shown in Fig. 2a and Supplementary Data 1, genes upregulated twofold or more in the MDA-MB-231, MDA-MB-361 and MDA-MB-157 cell lines, when compared to MDA-MB-468, were selected as candidate genes. Among the 253 genes identified, 16 encoding membrane or membrane-associated proteins were selected for further study as these receptors/transporters may interact with diffusible factors from adipocytes (Table 1). Q-PCR analysis confirmed that the expression profiles of these genes were consistent with the results from the cDNA microarray (Fig. 2c and Supplementary Fig. 2A). Next, we individually depleted each of these 16 candidate genes in MDA-MB-157 cells by pooled RNAi knockdown to evaluate their roles in MGDAs-mediated tumour enhancement (Supplementary Fig. 2B). Depletion of ARMCX1, ENPP1, FRMD5, GPC6, GPR126, RFTN1 and MCT2 abolished the colony number increase induced by co-culture with MGDAs (Fig. 2b). However, only ARMCX1, ENPP1 and MCT2 showed a better correlation between phenotype and expression level as high expression of these three genes was detected in MCF7, MDA-MB-361, MDA-MB-231 and MDA-MB-157 cells (for which MGDAs had a promoting activity) while low expression was found in MDA-MB-468 and SKBR3 cells (for which MGDAs co-culture had no significant effect) (Fig. 2c,d).

To further verify that ARMCX1, ENPP1 and MCT2 contribute to MGDAs-mediated effects, we depleted ARMCX1, ENPP1 and MCT2 expression in two additional cell lines (MCF7 and MDA-MB-231) and obtained similar results (Fig. 3a,b). However, we also observed that depletion of ARMCX1, ENPP1 and MCT2 inhibited the colony formation (Fig. 3b). To exclude the possibility that ARMCX1, ENPP1 and MCT2 are essential for breast cancer growth regardless of the MGDAs, we tested whether colony formation was enhanced by MGDAs in MDA-MB-468 and SK-BR3 cells ectopically expressing these genes (Fig. 3a). As shown in Fig. 3c only MCT2, but not ARMCX1 nor ENPP1, consistently enhanced colony formation in both MDA-MB-468 and SK-BR3 cells. Subcutaneous co-injection of MCT2-depleted MDA-MB-231 cells or MCT2-overexpressing MDA-MB-468 cells
with MGDAs in NOD/SCID/γnull mice reduced or accelerated tumour growth compared to the control groups, respectively (Fig. 3d,e). Taken together, these results from loss- and gain-of-function analyses suggest that the presence of MCT2 in breast cancer cells mediates the tumorigenic effect promoted by MGDAs.

**β-hydroxybutyrate enhances malignancy of breast cancer cells.** Based on the design of the in vitro co-culture system, the effects from MGDAs are most likely mediated by secreted soluble factors. Although adipocytes secrete many adipokines, a systematic analysis of factors from MGDAs is necessary. We collected and fractioned MGDAs-conditioned medium (CM) based on molecular weight into <10 kD, 10–50 kD and >50 kD fractions via selected filters and centrifugation. Only the <10 kD fraction stimulated colony formation when compared to cell culture medium (not MGDAs-CM) in the soft agar colony formation assay using MDA-MB-231 and MCF7 cells (Fig. 4a and Supplementary Fig. 3A). However, treatment with the same fraction did not enhance colony formation of MDA-MB-468, a non-responsive cell line. Furthermore, depletion or ectopic expression of MCT2 in MDA-MB-231, MCF7 and MDA-MB-468 cells, respectively, either abrogated or increased the response to the treatment with the <10 kD fraction (Fig. 4b and Supplementary Fig. 3B). These results strongly suggest that the <10 kD fraction of the MGDAs-CM contains at least one diffusible factor that exerts its effect through MCT2.

**Figure 1** | Mammary gland-derived adipocytes promote tumour growth in several breast cancer cell lines. (a) Soft agar colony formation assays using a panel of breast cancer cell lines co-cultured with MGDAs. (b) Tumour growth assays in NOD/SCID/γnull mice. Breast cancer cells were subcutaneously injected in both flanks of each mouse w/ or w/o MGDAs and tumour volumes were measured every 7 days. Four mice (n = 4) were used for each group. (c) Co-injection of MGDAs in xenograft models increased the tumour weights in several breast cancer cell lines. (d) MGDAs co-injection failed to enhance the tumour growth of MDA-MB-468 cells in mouse tumorigenicity assays. The experiment in a was performed in technical triplicate and repeated at least twice with similar results. In a-d, data show means ± s.d. *P < 0.05 (Student’s t-test).
MCT2 is a monocarboxylate transporter for several monocarboxylic acids, including pyruvate, lactate and β-hydroxybutyrate, which functions to move these molecules from the extracellular space into cells\textsuperscript{16,17}. Based on the fact that only the <10 kD fraction promoted colony formation in an MCT2-dependent manner, and that MCT2 is mainly for monocarboxylic acid transport, it is likely that one or more of the three small molecules mentioned above is essential for the observed effect of the MGDAs-CM on tumour malignancy.

To evaluate this possibility, we first compared the levels of pyruvate, lactate and β-hydroxybutyrate secreted from MGDAs with the levels from the corresponding stromal vascular fraction (SVF) cells, the second major cell type present in human breast adipose tissue. Although pyruvate, lactate and β-hydroxybutyrate were detected in CM collected from both MGDAs and SVF cells, the levels of β-hydroxybutyrate were significantly higher in the MGDAs-CM than in the corresponding SVF cells (Fig. 4c). To test the potential roles of pyruvate, lactate and β-hydroxybutyrate in promoting tumour malignancy, these small molecules were supplemented in colony formation assays for the MCT2-knockdown MDA-MB-231 and MCT2-overexpressing MDA-MB-468 cells, respectively. Interestingly, we observed that β-hydroxybutyrate (Fig. 4d) and lactate (Supplementary Fig. 3C) enhanced colony formation of MDA-MB-231 cells, but not MCT2-knockdown MDA-MB-231 cells. Supplementing with β-hydroxybutyrate, but not pyruvate or lactate, was also observed to significantly enhance colony formation in MCT2-overexpressing MDA-MB-468 cells (Fig. 4d and Supplementary Fig. 3C). Consistent with previous report that pyruvate was important for supporting proliferation of breast cancer cells\textsuperscript{18}, deprivation of pyruvate dramatically abolished the colony formation (Supplementary Fig. 3D), suggesting that pyruvate was also essential for supporting the growth of breast cancer cell in anchorage-independent condition. Since the colony formation of MCT2-expressing breast cancer cells was enhanced when co-culturing with MGDAs in the medium containing 1 mM pyruvate as shown in Figs 1 and 3, simply supplementing with higher amounts of pyruvate did not further promote colony formation (Supplementary Fig. 3C), pyruvate was less likely playing a key role in promoting colony

---

**Figure 2 | Identification of membrane proteins involved in MGDAs-mediated enhancement of tumorigenic activity.** (a) Summary of cDNA microarray analyses. In all, 253 genes were identified with at least twofold upregulation in MDA-MB-231, MDA-MB-361 and MDA-MB-157 compared to MDA-MB-468 cells. (b) RNAi knockdown screening of candidate genes involved in MGDAs-mediated tumorigenic activity promotion in MDA-MB-157 cells by soft agar colony formation assay. shLacZ was used as a knockdown control. (c) Expression profiles of ARMCX1, ENPP1, FRMD5, GPC6, GPR126, RFTN1 and MCT2 in six different breast cancer cell lines. The experiment was performed in technical triplicate. (d) Western blot analyses reconfirmed the expression profiles of ENPP1 and MCT2 in breast cancer cell lines (no commercial workable ARMCX1 antibody was found). The experiment in b was performed in technical triplicate and repeated at least twice with similar results. Data show means ± s.d. *P < 0.05 (Student’s t-test).
Taken together, these results suggest that promotion of breast cancer progression by MGDAs involves the secretion of adipocytes-CM (adipocytes-CM). Interestingly, the secretion of adipocytes-CM is responsible for promoting breast cancer progression. The dynamic range of β-hydroxybutyrate at 340 nm (OD 340 nm). The dynamic range of β-hydroxybutyrate is likely resulted from β-hydroxybutyrate treatment for 1 h, but not LCN2 (Fig. 5e). Consistently, the upregulation of LCN2 occurred later than IL-1β (Fig. 5f), suggesting that the induction of LCN2 expression may not be a direct response to β-hydroxybutyrate treatment. Taken together, these results suggest that promotion of breast tumorigenicity by β-hydroxybutyrate is likely resulted from upregulation of tumour-promoting genes through epigenetic modification of chromatin.

### Table 1 | List of membrane or membrane-associated candidate genes.

| Gene name | MB361/MB468 | MB231/MB468 | MB157/MB468 | Log2 ratio |
|-----------|-------------|-------------|-------------|------------|
| ABCG2     | 2.3         | 4.4         | 6.3         |            |
| AREG      | 2.0         | 4.4         | 4.0         |            |
| ARMEX1    | 6.8         | 6.6         | 6.9         |            |
| ATP2B1    | 2.6         | 2.9         | 2.3         |            |
| ENPP1     | 4.0         | 2.8         | 4.6         |            |
| FRAS1     | 2.9         | 2.7         | 2.6         |            |
| FRMD5     | 4.4         | 4.0         | 1.7         |            |
| GP56      | 4.5         | 5.4         | 3.2         |            |
| GPR126    | 1.6         | 1.8         | 1.8         |            |
| IL6R      | 1.0         | 2.4         | 2.4         |            |
| OSBPL8    | 1.8         | 2.2         | 1.9         |            |
| RAB8B     | 3.4         | 3.4         | 1.4         |            |
| RFTN1     | 5.8         | 5.7         | 2.8         |            |
| RHOF      | 3.2         | 3.2         | 2.0         |            |
| MCT2      | 5.1         | 5.3         | 6.2         |            |
| THBD      | 5.0         | 4.6         | 4.1         |            |

β-hydroxybutyrate alters expression profiles through HDAC.

Interestingly, β-hydroxybutyrate was recently recognized as an endogenous inhibitor of class I HDACs (ref. 19). Treatment with β-hydroxybutyrate increases global histone acetylation, such as at histone H3 Lys9 and Lys14 (H3K9 and H3K14), which in turn results in gene activation. To test if β-hydroxybutyrate has a role in altering global histone acetylation of MCT2-expressing breast cancer cells, we treated MDA-MB-231, MCF7 and MDA-MB-157 cells, which all express MCT2, and the corresponding cells depleted with MCT2 with β-hydroxybutyrate. As shown in Fig. 5a and Supplementary Fig. 5A, the level of H3K9 acetylation was enhanced in a dose- and time-dependent manner, while such enhancement was not observed in MCT2-depleted cells. Consistently, when we co-cultured MDA-MB-231 cells with MGDAs, the acetylation of H3K9 was also increased (Supplementary Fig. 5B), arguing that the tumorigenic effect of β-hydroxybutyrate is likely mediated by epigenetic histone modification followed by upregulation of tumour-promoting genes.

To identify which genes were induced by β-hydroxybutyrate in an MCT2-dependent manner, we performed a subtractive microarray analysis as shown in Fig. 5b. About 34 genes in MDA-MB-231 cells treated with β-hydroxybutyrate were found to be upregulated in an MCT2-dependent manner. By performing gene ontology analysis using the Biological Process (BP) GO term, these genes were classified into six groups (Fig. 5c). Intriguingly, most genes on the list were highly expressed in breast cancers compared to normal mammary tissue (data were adapted from Oncomine database, https://www.oncomine.org/resource/login.html) (Table 2). The genes belonging to ‘Cell growth and proliferation’ and ‘Apoptosis related pathway’ were selected for further validation by Q-PCR analysis. Among these genes, IL-1β and LCN2 were the most upon β-hydroxybutyrate treatment (Fig. 5d). Furthermore, the expressions of IL-1β and LCN2 were also induced in breast cancer cells co-culture with MGDAs (Supplementary Fig. 5c). To test whether the upregulation of IL-1β and LCN2 is attributed to histone H3K9 acetylation, we performed chromatin immunoprecipitation (ChIP) analysis of the IL-1β and LCN2 promoters with six primer pairs of primers spanning each promoter region. It was noted that histone H3K9 acetylation of the IL-1β was immediately increased after β-hydroxybutyrate treatment for 1 h, but not LCN2 (Fig. 5e). Consistently, the upregulation of LCN2 occurred later than IL-1β (Fig. 5f), suggesting that the induction of LCN2 expression may not be a direct response to β-hydroxybutyrate treatment. Taken together, these results suggest that promotion of breast tumorigenicity by β-hydroxybutyrate is likely resulted from upregulation of tumour-promoting genes through epigenetic modification of chromatin.

IL-1β and LCN2 contribute to MGDAs-mediated malignancy.

The roles of IL-1β and LCN2 in breast cancer progression have been reported. To examine their roles on promoting colony formation mediated by MGDAs, we added recombinant IL-1β and LCN2 proteins in soft agar colony formation assays, and found that either of them increased colony numbers in a dose-dependent manner (Fig. 6a). On the other hand, knockdown of either IL-1β or LCN2 in MDA-MB-231 only slightly abrogated MGDAs-mediated colony promotion (Supplementary Fig. 6A). Although depletion of both IL-1β and LCN2 abolished the MDGAs-mediated colony promotion of MDA-MB-231 breast cancer cells, it also caused severe inhibition of cell growth (Supplementary Fig. 6B). To avoid the severely retarded growth effect caused by the double knockdown, we used IL-1β-neutralizing antibody to treat LCN2-depleted MDA-MB-231 breast cancer cells for evaluating the colony formation. As shown in Fig. 6b, adding IL-1β antibody further reduced the colony number of LCN2-depleted MDA-MB-231 breast cancer cells co-cultured with MGDAs, suggesting that both IL-1β and LCN2 contribute to MGDAs-mediated tumour malignancy. Based on the above results, a novel molecular mechanism linking adipocytes and breast cancer progression via MCT2 was established. The heterotypic cell-cell interaction within the breast cancer microenvironment upregulated IL-1β/LCN2 expressions in breast cancer cells, which in turn markedly enhanced breast cancer tumour progression (Fig. 6c).
(ΔCt = -7.84) was used as a cut-off value based on a receiver operating characteristic curve analysis to define ‘high or low’ expression of MCT2. Kaplan-Meier (KM) analysis showed that patients with high MCT2 expression had a shorter survival time compared to patients with low MCT2 expression, n = 106 (Fig. 7a, P = 0.038, log-rank test). The association of MCT2 expression with poor prognosis was also significant even after adjustment for age, tumour size, lymph node status, grade and ER expression (Table 3). These results suggest that MCT2 is an independent prognostic factor in patients with breast cancer. To validate this result, another independent breast cancer cohort with larger sample size (n = 327, Supplementary Tables 3 and 4, data adapted from the breast tumour gene expression profiling by Kao et al.22
was used for further survival analysis. As shown in Supplementary Fig. 7A, KM analysis showed that patients with high MCT2 expression had a shorter survival time compared to patients with low MCT2 expression ($P = 0.003$, log-rank test). Furthermore, expression of MCT2 in breast ductal carcinoma was increased when compared to normal mammary tissues (Supplementary Fig. 7B, data adapted from the breast tumour gene expression profiling by Richardson et al.23). In addition, we have validated the specificity of MCT2 antibody in immunohistochemical staining as shown in Supplementary Fig. 8. We then performed examination of MCT2 expression in another independent cohort of 36 breast cancer specimens. Breast cancer patients with high MCT2 expression correlated with poor prognosis (Fig. 7b,c, 22% of the cases were MCT2 positive, $P = 0.021$, log-rank test). Furthermore, the expressions of IL-1β and LCN2 mRNA were positively correlated with MCT2 expression in breast cancer specimens.

In addition, we have validated the specificity of MCT2 antibody in immunohistochemical staining as shown in Supplementary Fig. 8. We then performed examination of MCT2 expression in another independent cohort of 36 breast cancer specimens. Breast cancer patients with high MCT2 expression correlated with poor prognosis (Fig. 7b,c, 22% of the cases were MCT2 positive, $P = 0.021$, log-rank test). Furthermore, the expressions of IL-1β and LCN2 mRNA were positively correlated with MCT2 expression in breast cancer specimens.
Figure 5 | Treatment of β-hydroxybutyrate induces changes of gene expression profiles through epigenetic effects. (a) The level of H3K9 acetylation in MCT2-depleted MDA-MB-231 cells was assessed upon β-hydroxybutyrate treatment using a variety of doses and times. The experiment was repeated at least twice with similar results. (b) Summary of cDNA microarray analyses. Thirty-four genes were identified with at least 1.5-fold upregulation in β-hydroxybutyrate-treated MDA-MB-231 cells in an MCT2-dependent manner. (c) GO analysis of β-hydroxybutyrate-induced genes. The GO terms were classified into six groups and the percentages were shown. (d) Q-PCR analyses confirmed the expression levels of IL-1β and LCN2 in MDA-MB-231 cells treated with β-hydroxybutyrate. (e) Chromatin immunoprecipitation of acetylated histone H3K9 on IL-1β and LCN2 proximal promoter regions in MDA-MB-231 cells upon 20 mM β-hydroxybutyrate treatment for 1 h. (f) IL-1β and LCN2 expression levels in MDA-MB-231 cells treated with 20 mM β-hydroxybutyrate for various times. The experiments in d–f were performed in technical triplicate and repeated at least twice with similar results. Data show means ± s.d. *P<0.05 (Student’s t-test).

Table 2 | List of β-hydroxybutyrate-induced genes for each GO term.

| Term                              | Genes (ranked by induction fold) |
|-----------------------------------|----------------------------------|
| Signalling transduction           | IL-1β*, IL6*, LCN2*, ISG20*, PIK3CD*, TNIP2*, CXCL1, IL11*, WNT5B*, ANKRD1*, EIF2C4* |
| Cell growth and proliferation     | IL-1β*, SOD2, IL6*, ISG20*, LEPREL2*, GATA2*, CXCL1, IL11* |
| Metabolic process                 | IL-1β*, ADA*, ISG20*, KYNU*, ANKRD1*, TUB2B |
| Apoptosis-related pathway         | IL-1β*, OLR1*, SOD2, IL6*, ADA*, LCN2*, TNIP2*, TREXI*, ANKRD1* |
| Oxidative stress response         | IL-1β*, OLR1*, SOD2, IL6*, ADA*, LCN2* |

Asterisks indicated that genes were highly expressed in breast tumour compared to normal mammary tissue (data were adapted from Oncomine database, https://www.oncomine.org/resource/login.html).
The correlation between MCT2 expression and poor prognosis in breast cancer was further strengthened when combined with either high expression of IL-1β or LCN2 (cut-off value of 7.34 for IL-1β and 7.42 for LCN2, respectively) (Fig. 7e). These findings suggest that high expression of MCT2, in combination with IL-1β or LCN2 expression, may serve as an important clinical biomarker for poor prognosis in breast cancer patients.

**Discussion**

It has been shown that larger breast size is associated with an increased risk of breast cancer progression. This correlation is even more significant in the study of decreased cancer risk by breast reduction. Recent work further demonstrates that breast size is highly correlated with breast cancer mortality even after adjustment for BMI (ref. 25), suggesting that local adiposity in breast tissue may have a significant impact on cancer progression. Here, we find that the monocarboxylate transporter protein MCT2, expressed in a distinct subtype of breast cancer cells, plays a pivotal role in the interplay with MGDAs. We show that MGDAs promote the tumorigenicity of MCT2-expressing breast cancer cells in a paracrine manner. β-hydroxybutyrate secreted from MGDAs can be transported into breast cancer cells via MCT2. Intracellular β-hydroxybutyrate functions as a class I HDAC inhibitor and induces the expression of tumour-promoting genes through epigenetic modification, leading to tumorigenicity enhancement.

(Fig. 7d). The correlation between MCT2 expression and poor prognosis in breast cancer was further strengthened when combined with either high expression of IL-1β or LCN2 (cut-off value of −ΔCt: −7.34 for IL-1β and −7.42 for LCN2, respectively) (Fig. 7e). These findings suggest that high expression of MCT2, in combination with IL-1β or LCN2 expression, may serve as an important clinical biomarker for poor prognosis in breast cancer patients.

**Figure 6 | IL-1β and LCN2 play important roles in MGDAs-mediated tumorigenicity enhancement.** (a) MDA-MB-231 cells were supplemented with recombinant IL-1β and LCN2 in soft agar colony formation assays. (b) Soft agar colony formation assays showed that knockdown of LCN2 combining IL-1β neutralizing antibody treatment (2 μL-β, 1 μg ml⁻¹) significantly abrogated the increase of colonies induced by MGDAs co-culture with MDA-MB-231 cells. Mouse IgG (1 μg ml⁻¹) was used as a control. The experiments in a,b were performed in technical triplicate and repeated at least twice with similar results. Data show means ± s.d. *P<0.05 (Student’s t-test). (c) Schematic shows the heterotypic interaction between MGDAs and MCT2-expressing breast cancer cells in the breast tissue microenvironment. MGDAs promote the tumorigenicity of MCT2-expressing breast cancer cells in a paracrine manner. β-hydroxybutyrate secreted from MGDAs can be transported into breast cancer cells via MCT2. Intracellular β-hydroxybutyrate functions as a class I HDAC inhibitor and induces the expression of tumour-promoting genes through epigenetic modification, leading to tumorigenicity enhancement.
Since MGDAs promoted tumour malignancy regardless of the  
IL-6R/IL-6 signalling mediates the promotion of tumour malignancy by adipocytes.

MGDAs, indicating that an unknown pathway distinct from the aromatase, a rate-limiting enzyme for estradiol synthesis. MCT1 was observed in several cancers, such as breast and primary MGDAs diminished MGDAs-mediated promotion of malignancy in an MCT2-dependent manner (Figs 4 and 5). While it could not be directly determined whether breast adipocytes secrete \( \beta \)-hydroxybutyrate specifically to promote tumour malignancy, it was reported that elevated expression of monocarboxylate transporters (MCTs) may have relevance to tumour progression. In addition to adipokines, an elevated level of oestrogen was thought to be the major factor for ER\( \beta \)-positive breast cancer risk in postmenopausal women. When ovarian production of female steroid hormones decreases, adipose tissue becomes an important source for oestrogen production due to the expression of aromatase, a rate-limiting enzyme for estradiol synthesis. Since MGDAs promoted tumour malignancy regardless of the ER\( \beta \) status of the tested cells (Fig. 1), the oestrogen level does not appear to be the determining factor. Interestingly, adipocytes secrete \( \beta \)-hydroxybutyrate, which promotes tumour malignancy in an MCT2-dependent manner (Figs 4 and 5). While it could not be directly determined in vivo that breast adipocytes secrete \( \beta \)-hydroxybutyrate specifically to promote tumour malignancy, our results that depletion of \( \beta \)-hydroxybutyrate present in primary MGDAs diminished MGDAs-mediated promotion of tumorigenic activity in vitro (Supplementary Fig. 4) suggest that \( \beta \)-hydroxybutyrate either from breast adipocytes or other source promotes tumorigenic activity. It was reported that elevated expression of monocarboxylate transporters (MCTs) may have relevance to tumour progression. MCT1 was observed in several cancers, such as breast and...
colorectal cancers30,31, while MCT2 and MCT4 were highly expressed in prostate cancer and renal cell carcinoma, respectively32,33,34. Intriguingly, MCT2, but not MCT1, played an important role in MGDAs-mediated promotion of tumorigenic activity as observed above. Several lines of evidence supported this notion; co-culturing with MGDAs and treatment of β-hydroxybutyrate shown in Figs 1 and 3 failed to promote the colony numbers of MDA-MB-468 cells, which expressed relatively higher level of MCT1 (Supplementary Fig. 9A). Furthermore, ectopic expression of MCT1 failed to rescue MCT2-depleted MDA-MB-231 cells in response to β-hydroxybutyrate treatment (Supplementary Fig. 9B,C). MCT2 showed higher affinity (about tenfold) to most monocarboxylates than MCT1 (ref. 34). Although the biological difference between MCT1 and MCT2 in response to β-hydroxybutyrate is evident, the detailed mechanism to explain this difference deserves further exploration.

MCT2 has been reported to locate in mitochondria with a role for mitochondrial metabolism35, and inhibition of MCT2 suppresses colorectal cancer progression via induction of mitochondrial dysfunction36. However, our results revealed that co-culture of MGDAs with breast cancer cells increased tumour malignancy in a paracrine and MCT2-dependent manner, suggesting that the major role of MCT2 is likely for the function across cell membrane of cancer cells. Through immunofluorescence staining analysis, a membrane-staining pattern of MCT2 in breast cancer cells was revealed (Supplementary Fig. 10). Consistently, membrane staining of MCT2 was correlated with poor prognosis in breast cancer patients (Fig. 7b,c).

Increase of the circulating level of β-hydroxybutyrate through supplementation or ketogenic diet was reported to prolong survival of mice with brain metastatic tumour37,38. At the same setting, the authors also found that decreased blood glucose was also correlated with longer survival in brain tumour bearing mice. Thus, it was not clear whether decrease of blood glucose or increase of β-hydroxybutyrate contributed to tumour reduction. Several experiments showed that ketogenic diet without calories restriction and blood glucose decrease did not reduce tumour growth39–41. These results suggest that reduction of blood glucose may be the key factor to restrict tumour progression in vivo despite the presence of high level of circulating ketone bodies. We have observed here that addition of β-hydroxybutyrate promoted breast tumour growth in an MCT2-dependent manner in a mouse xenograft model. In those mice fed with regular chow diet ad libitum, the blood glucose level was not changed (Supplementary Fig. 11). This finding reflects a delicate role of the metabolite balance in tumour progression. Although ketone supplement reportedly decreased brain tumour cell viability in vitro38, treatment of β-hydroxybutyrate did not reduce proliferation in breast cancer cells (Supplementary Fig. 12), suggesting that brain and breast cancer cells have different response to β-hydroxybutyrate. Unlike brain and muscle cells42, breast cancer cells could not use β-hydroxybutyrate as alternative energy source (Supplementary Fig. 13). Thus, β-hydroxybutyrate may serve as HDAC inhibitor, as reported recently39, to modulate gene expression in breast cancer cells.

Treatment of HDAC inhibitors globally increases the acetylation of histones H3 and H4 (ref. 43), which is generally associated with transcription activation and gene upregulation44. Although the HDAC inhibitors are considered as promising agents for certain haematological tumours treatment, the efficacy in treating solid tumours is limited45. It is likely that the accessibility of the HDAC inhibitors to these two kinds of malignancy may explain the discrepancy. In fact, treatment of low and non-cytotoxic dosages of HDAC inhibitors preferentially induces epithelial-to-mesenchymal transition and proliferation genes expression46–48. Our observation that treatment of cancer cells expressing MCT2 with β-hydroxybutyrate, a weak HDAC inhibitor, increased acetylation of histones, and upregulated expressions of IL-1β and LCN2 (Figs 5 and 6), is consistent with low dose effect of HDAC inhibitor as reported46–48. Furthermore, the significant correlation between MCT2 and IL-1β/LCN2 expressions in clinical breast cancer specimens supports the role of an MCT2-mediated oncogenic pathway in breast cancer progression. Thus, our findings provide the mechanistic basis for a novel interaction between breast tissue adipocytes and breast tumour. Further research into this detailed mechanism may offer new insights for better diagnostic and prognostic assessments, and for new therapeutic opportunities.

**Methods**

**Ethics statement.** Human samples were obtained from National Taiwan University Hospital (NTUH) and Wan Fang Hospital, Taipei Medical University. The samples were encoded to protect patient confidentiality. All patients signed an informed consent, which was approved by the Institutional Review Board of Human Subjects Research Ethics Committee of National Taiwan University, Taipei, Taiwan (IRB no. 99002001R). The experiments were performed in technical triplicate and repeated at least twice independently. Similar results were observed and one representative result was shown.

**Cell culture.** Human breast cancer cell lines MCF7, MDA-MB-157, MDA-MB-231, MDA-MB-361, MDA-MB-468 and SKBr3 were obtained from American Type Culture Collection and cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mm glutamine, 50 μg/mL penicillin, 100 unit/mL streptomycin, and antibiotics/antimycotics (IRB no. WFH-IRB-99049). Clinical information was obtained from pathology reports, and the characteristics of these cases are given in Supplementary Tables 1 and 2. Patients with at least 5 years follow-up were included in this study. The animal studies were approved by the Institutional Animal Care and Use Committee of the Academia Sinica, Taipei, Taiwan (Protocol # 14-05-708). The experiments were performed in technical triplicate and repeated at least twice independently. Similar results were observed and one representative result was shown.

**Statistical methods.** Except for the clinical correlation and quantification for specific immunoablots, all data were presented as mean ± s.d., and Student’s t-test (two-tailed, unpaired) was used to compare control and treatment groups. *P*-indicated statistical significance with *P* < 0.05. Data distribution was assumed to be normal. The variance was similar between the groups that were being statistically compared (by F-test). We chose our sample sizes based on those commonly used in this field without predetermination by statistical methods. The following analyses were performed using MedCalc statistics software. KM method was plotted and log-rank test was used to evaluate the statistical significance between patients with high and low MCT2 expression in survival. To determine the effects of different variables on overall survival, univariate Cox proportional-hazards regression analysis was performed. Multivariate Cox regression analysis was used to adjust the association between survival and MCT2 gene expression level for varying clinical parameters including age, tumour size, lymph-node status, tumour grade and ER expression. The optimal cut-off values of MCT2, IL-1β and LCN2 gene expression levels for 5-year survival were determined using receiver operating characteristic analysis. Pearson’s correlation was used to evaluate the correlation between MCT2, IL-1β and LCN2 gene expression levels.

**Preparation of primary mammary gland-derived adipocytes.** Human breast adipose tissue samples were collected from breast cancer mastectomy specimens in NTUH. Adipose tissue pieces were minced and immediately incubated with digestion buffer (Collagenase I (250 μg ml⁻¹, Sigma) dissolved in PBS containing 2% bovine serum albumin (Sigma))34. For the incubation, the tubes are closed tightly incubated in a 37°C incubator for 2 h. SVF was separated by centrifugation (5 min, 500 g, room temperature, 300g). Primary MGDAs were cultured in DMEM/F12 medium supplemented with 10% FBS and antibiotics/antimycotics. The purity of the isolated MGDAs was assessed by fluorescence-activated cell sorting analysis using the lipophilic fluorescent dye Nile red at a final concentration of 1 μg ml⁻¹ (Sigma, 1 mg ml⁻¹ DMSO stock, diluted 1:100 in PBS immediately before use) and nuclear stain DAPI (Sigma) at a final concentration of 1 μg ml⁻¹. 

**Soft agar colony formation assay.** Primary MGDAs (10–25 μl) mixed with 0.5% agar/complete DMEM/F12 growth medium were added into one well of 12-well plate to form a base layer. After the agar was solidified, 1,500 breast cancer cells suspended in 0.35% agar/1% FBS DMEM medium were seeded
on top of bottom agar. Cells were maintained in a humidified 37°C incubator for 14 days and the colonies were fixed with ethanol containing 0.05% crystal violet for quantitation. The plate was scanned for result determination experiment. MDA-MB-157 cells were transfected with shRNA (Taipei, Taiwan). The target sequences of shRNA were listed in Supplementary Table 7. MDA-MB-157, MDA-MB-231 and MCF7 cells were transfected with shRNA (Bio-Rad) before loading and verified by H3 (#4499, Cell Signaling Technology), anti-MCT1 (AB3538P, Merck Millipore) and 1:1,000 dilution of anti-ENPP1 (GTX103447, GeneTex), anti-fibronectin (GTX72360, GeneTex). The intensity of western blot bands was quantified using ImageJ software (NIH). The uncropped scans of immuno-blots can be found in Supplementary Figs 14 and 15.

Immunoblotting. Whole-cell extracts were prepared by scraping in RIPA lysis buffer (50 mM Tris-HCl (pH, 7.4), 150 mM NaCl, 1% NP-40, 1% Triton X-100, 2 mM EDTA, 50 mM NaF, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM PMSF and 1 x protease inhibitor cocktail (complete, Roche)) followed by centrifugation at 13,000 r.p.m. at 4°C. Immunoblot analysis was performed after 10 or 15% SDS-PAGE, with overnight incubation of 1:200 dilution of anti-MCT2 (sc-50322, Santa Cruz), or 1:1,000 dilution of anti-ENPP1 (GTX103447, GeneTex), anti-acetyl-Histone H3 (Lys9) (9694, Cell Signaling Technology), anti-Histone H3 (#4499, Cell Signaling Technology), anti-MCT1 (AB3538P, Merck Millipore) antibodies followed by a 1:1,000 dilution of horseradish peroxidase-conjugated anti-mouse or anti-rabbit secondary antibody (GeneTex). Signals were detected using Immobilon Western Chemiluminescent HRP Substrate (Merck Millipore). Protein concentration was determined by the Bradford assay (Bio-Rad) before loading and verified by z-tubulin level using a 1:10,000 dilution of anti-z-tubulin antibody (GTX27360, GeneTex). The intensity of western blot bands was quantified using ImageJ software (NIH). The uncropped scans of immuno-blots can be found in Supplementary Figs 14 and 15.

Immunohistochemistry. Formalin-fixed paraffin embedded primary tumour tissue sections were stained by using the Ventana automated immunostainer Benchmark XT (Ventana Medical Systems). Homemade mouse anti-MCT2 monoclonal antibody (1 µg µl−1) was diluted to 1:200, and incubated at room temperature for 2 h. Signals were detected using the OptiView DAB IHC Detection Kit (Ventana Medical Systems). All slides were counterstained with haematoxylin, and the images were taken using an Aperio Digital Pathology System. Samples were identified as MCT2-positive if more than 5% of the tumour cells were positive for membrane staining. The systemic administration of recombinant IL-1b was performed by intraperitoneal injection. The tumours were measured using an electronic caliper every 4 days. The mice were treated with 1% formaldehyde for 10 min at room temperature. Cross-linking reaction was stopped by the addition of 0.125 M glycine (final concentration). Cryosections (10 µm) were collected in cold PBS containing 1 mM PMSF. Cells were resuspended in ChIP Swelling buffer (5 mM PIPES (pH, 8.0), 85 mM KC1, 1% NP-40, 1% Triton X-100) and incubated at 4°C for 20 min. Then the nuclei pellet collected by centrifugation was resuspended in Nuclei Lysis buffer (50 mM Tris-HCl (pH, 8.0), 10 mM EDTA, 1% SDS and 1 mM PMSF) and sonicated to an average size of 500 bp. After sonication and centrifugation (500 g, 15 min), the nuclei at 13,000 r.p.m. for 15 min at 4°C, the supernatant was added ChIP dilution buffer (16.7 mM Tris-HCl (pH, 8.0), 167 mM NaCl, 1.2 mM EDTA, 0.01% SDS, 1.1% Triton X-100 and 1 mM PMSF). IPs were performed with anti-aciethyl-Histone H3 (Lys9) (9694, Cell Signaling Technology), anti-Histone H3 (ab7917, Abcam) and rabbit IgG control (sc-2027, Santa Cruz Biotechnology) antibodies and incubated at 4°C overnight. The beads were washed twice and eluted by 1% SDS elution buffer (1% SDS, 0.1 M NaHCO3, and 0.01 mg ml−1 herring sperm DNA). After treatment with Proteinase K, DNA was extracted with phenol-chloroform, precipitated with ethanol and dissolved in Tris-EDTA buffer (10 mM Tris-HCl (pH, 8.0) and 1 mM EDTA). ChIP products and input DNA were analysed by Q-PCR. Primers spanning the promoter regions of the human IL-1b and LCN2 genes are shown in Supplementary Table 8.

Immunofluorescence staining. MDA-MB-231 and MDA-MB-468 cells were seeded on coverslips for immunofluorescence staining. The cells were washed with PBS, fixed in 4% parafomaldehyde for 10 min and permeabilized in 0.2% Triton X-100 for 15 min at room temperature. After washing with PBS, the cells were blocked with PBS containing 10% FBS for 30 to 60 min, and incubated with homemade mouse anti-MCT2 monoclonal antibody (1:12,000 overnight) at 4°C. The cells were washed with PBS and incubated with fluorochrome-conjugated secondary antibody (1:500, Alexa Fluor 488 goat-anti-mouse, Invitrogen) for 1 h. The coverslips with stained cells were then washed, stained with DAPI (1:1,000) and mounted onto glass slides with mounting medium and examined by Leica TCS-SP5-MP-SMD confocal microscope.

Mitochondrial respiration analysis. The mitochondrial bioenergetics profiles in breast cancer cells were performed using a Seahorse XF96 Cell Mito Stress Test Kit (Seahorse Biosciences) according to instructions from the manufacturers. MDA-MB-231 cells were seeded at 20,000 cells per well (96-well Seahorse cell culture plate) in pyruvate-free condition and incubated overnight. On the day of assay, the medium was replaced with 175 µl of Seahorse assay medium supplemented with different concentrations of pyruvate or b-hydroxybutyrate and then incubated for 1 h at 37°C without CO2. The oxygen consumption rate (OCR) was determined by Seahorse XF96 analyser (Seahorse Biosciences). During the period of OCR measurement, sequential injection of 1 µM oligomycin, 0.5 µM carboxy cyanide p-trifluoromethoxyphenyl hydrazone (FCCP) and 0.5 µM carbonyl cyanide m-trifluoromethoxyphenyl hydrazone (rotenone/antimycin were used to determine the mitochondrial respiration parameters including basal OCR, maximal OCR and reserve respiratory capacity.

Data availability. Gene expression microarray data of b-hydroxybutyrate treatment were deposited in the Gene Expression Omnibus (GEO) database with an accession number of GSE84053. The Oncomine dataset used in the current study are available in a public repository from the website (https://www.oncomine.org/resource/login.html). The authors declare that all the other data supporting the findings of this study are available within the article and its Supplementary information files and from the corresponding author on reasonable request.

References
1. Liotta, L. A. & Kohn, E. C. The microenvironment of the tumour-host interface. Nature 411, 375–379 (2001).
2. Rong, S. et al. Tumorigenicity of the met proto-oncogene and the gene for hepatocyte growth factor. Mol. Cell. Biol. 12, 5152–5158 (1992).
3. Orimo, A. et al. Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDH. Proc. Natl. Acad. Sci. USA 102, 135–140 (2005).

4. Tyan, S. W. et al. Breast cancer cells induce cancer-associated fibroblasts to secrete hepatocyte growth factor to enhance breast tumorigenesis. PLoS ONE 6, e15313 (2011).

5. Chen, J. et al. CCL18 from tumor-associated macrophages promotes breast cancer metastasis via PITPNM3. Cancer cell 19, 541–555 (2011).

6. Wang, Y. Y. et al. Adipose tissue and breast epithelial cells: a dangerous dynamic duo in breast cancer. Cancer Lett. 342, 142–151 (2012).

7. Kimijima, I., Ohtake, T., Sagara, H., Watanabe, T. & Takeno, S. Scattered fat invasion: an indicator for poor prognosis in premenopausal, and for positive estrogen receptor in postmenopausal breast cancer patients. Oncology 59 Suppl 1, 25–30 (2000).

8. Yamaguchi, I., Ohtani, H., Nakamura, K., Shimokawa, K. & Kanematsu, T. Prognostic impact of marginal adipose tissue invasion in ductal carcinoma of the breast. Am. J. Clin. Pathol. 130, 382–388 (2008).

9. Vona-Davis, L. & Rose, D. P. Adipokines as endocrine, paracrine, and autocrine factors in breast cancer risk and progression. Endocr. relat. cancer 14, 189–206 (2007).

10. D’Esposito, V. et al. Adipocyte-released insulin-like growth factor-1 is regulated by glucose and fatty acids and controls breast cancer cell growth in vitro. Diabetologia 55, 2811–2822 (2012).

11. Surmacz, E. Obesity hormone leptin: a new target in breast cancer? Histopathology 34, 153–162 (1999).

12. Dirat, B. et al. Carcinosarcoma in rats. Br. J. Cancer 52, 87–92 (1985).

13. Fierro, K. C., Tsidale, M. J., Preston, T., Plumb, J. A. & Calman, K. C. Failure of systemic ketosis to control cachexia and the growth rate of the Walker 256 carcinosarcoma in rats. Br. J. Cancer 52, 1711–1720 (2014).

14. Fei, R. & Menon, K. V. Ketogenic diet and hypoxia activates SDF-1/CXCL12 secretion. Cell 135, 1771–1782 (2009).

15. Zhou, W. et al. The calorically restricted ketogenic diet, an effective alternative therapy for malignant brain cancer. Nutr. Metab. (Lond) 4, 5 (2007).

16. Newman, J. C. & Verdin, E. Ketone bodies as signaling metabolites. Trends Endocrinol. Metab. 25, 42–52 (2014).

17. Drogaris, P. et al. Histone deacetylase inhibitors globally enhance h3/h4 tail acetylation without affecting h3 lysine 56 acetylation. Sci. Rep. 5, 220 (2015).

18. Hier, J. et al. Histone deacetylase inhibitor induction of epithelial-mesenchymal transitions via up-regulation of Snail facilitates cancer progression. Biochim. biophys. acta 1833, 663–671 (2013).

19. Kon, D. et al. Histone deacetylase inhibitors induce epithelial-to-mesenchymal transition in prostate cancer cells. PLoS ONE 7, e45045 (2012).

20. Huang, G. M. et al. Histone deacetylase inhibitor induction of epithelial-mesenchymal transitions via up-regulation of Snail facilitates cancer progression. Biochim. biophys. acta 1833, 663–671 (2013).

21. Jansen, L. A., Backstein, R. M. & Brown, M. H. Breast size and breast cancer: a systematic review. J. Plast. Reconstr. Aesthet. Surg. 67, 1615–1623 (2014).

22. Vermeulen, S. et al. Stromal fibroblasts express systemic ketosis to control cachexia and the growth rate of the Walker 256 carcinosarcoma in rats. Br. J. Cancer 52, 87–92 (1985).

23. Fierro, K. C., Tsidale, M. J., Preston, T., Plumb, J. A. & Calman, K. C. Failure of systemic ketosis to control cachexia and the growth rate of the Walker 256 carcinosarcoma in rats. Br. J. Cancer 52, 87–92 (1985).

24. Storlacci, E. et al. The colorably restricted ketogenic diet, an effective alternative therapy for malignant brain cancer. Nutr. Metab. (Lond) 4, 5 (2007).

25. Newman, J. C. & Verdin, E. Ketone bodies as signaling metabolites. Trends Endocrinol. Metab. 25, 42–52 (2014).

26. Drogaris, P. et al. Histone deacetylase inhibitors globally enhance h3/h4 tail acetylation without affecting h3 lysine 56 acetylation. Sci. Rep. 5, 220 (2015).

27. Hier, J. et al. Histone deacetylase inhibitors induce epithelial-to-mesenchymal transition in prostate cancer cells. PLoS ONE 7, e45045 (2012).

28. Donohoe, D. R. et al. The Warburg effect dictates the mechanism of butyrate-mediated histone acetylation and cell proliferation. Mol. Cell 48, 612–626 (2012).

Acknowledgements
This work was supported by grants from Academia Sinica Peak Project (grant numbers 2371 and 4012) and National Science Council Most (grant number NSC102-2321-B-001-016), Taiwan. We thank Dr A Ball for his critical reading of the manuscript.

Author contributions C.-K.H. and P.-H.C. performed and conceived the experiments. C.-K.H. wrote the manuscript. W.-H.L. and K.-J.C. provided fresh clinical specimens. C.-L.C. and Y.-M.J. provided the paraffin-embedded tumour specimens and analysed the IHC results. J.-Y.S., C.-M.H and W.-H.L. coordinated the project and conceived the experiments.

Additional information
Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications

Competing financial interests: The authors declare no competing financial interests.

Reprints and permission information is available online at http://npg.nature.com/reprintsandpermissions/

How to cite this article: Huang, C.-K. et al. Adipocytes promote malignant growth of breast tumours with monocarboxylate transporter 2 expression via β-hydroxybutyrate. Nat. Commun. 8, 14706 doi: 10.1038/ncomms14706 (2017).

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.