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SHORT REPORT

Dexamethasone promotes breast cancer stem cells in obese and not lean mice

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Abstract

Obesity is highly prevalent in breast cancer patients and is associated with increased recurrence and breast cancer-specific mortality. Glucocorticoids (GC) are used as an adjuvant in cancer treatment and are associated with promoting breast cancer metastasis through activation of stemness-related pathways. Therefore, we utilized the synergetic allograft E0771 breast cancer model to investigate if treatment with GCs had differential effects on promoting cancer stem cells in lean and diet-induced obese mice. Indeed, both lean mice treated with dexamethasone and obese mice with no treatment had no effect on the ex vivo colony-forming ability, mammosphere formation, or aldehyde dehydrogenase (ALDH) bright subpopulation. However, treatment of obese mice with dexamethasone resulted in a significant increase in ex vivo colony formation, mammosphere formation, ALDH bright subpopulation, and expression of pluripotency transcription factors. GC transcriptionally regulated genes were not altered in the dexamethasone-treated groups compared to treatment controls. In summary, these results provide initial evidence that obesity presents a higher risk of GC-induced cancer stemness via non-genomic GC signaling which is of potential translational significance.

KEYWORDS
breast cancer, cancer stem cells, glucocorticoids, obesity, tumour initiating cells

1 | INTRODUCTION

Steroids are routinely used in patients with solid tumors as adjuvant therapy to manage tumor and treatment-related symptoms. However, a meta-analysis of >80 000 patients found that the use of steroids in solid tumors was associated with reduced overall and progression-free survival.[1] One explanation for this is the observation is that glucocorticoids (GCs) promote a stem cell phenotype.[2–4] In breast cancer, plasma levels of cortisol (an endogenous GC) increase during breast cancer progression, inducing activation of the glucocorticoid receptor (GR) resulting in increased metastatic colonization.[5] Worryingly, treatment with dexamethasone (a clinically used GC drug) also activated the GR causing increased metastatic burden and decreased survival.[5] Obesity affects more than half a billion adults worldwide and it is a well-known risk factor for breast cancer and an indicator of poorer prognosis.[6,7] Indeed, obese breast cancer patients have an increased relative risk of recurrence of 40% to 50%, however, the underlying biology behind the link between obesity and breast cancer progression remains unclear.[8] Obesity causes chronic inflammation in the adipose tissue and elevated

Abbreviations: ALDH, aldehyde dehydrogenase; GC, Glucocorticoids; GR, glucocorticoid receptor; HFD, high-fat diet.

levels of cortisol in both the local adipose tissue and the systemic circulation.[9,10] Given the role of GCs in breast cancer metastasis, we hypothesized that obesity may increase the risk of GC-induced breast cancer reoccurrence by further promoting cancer stem cells.

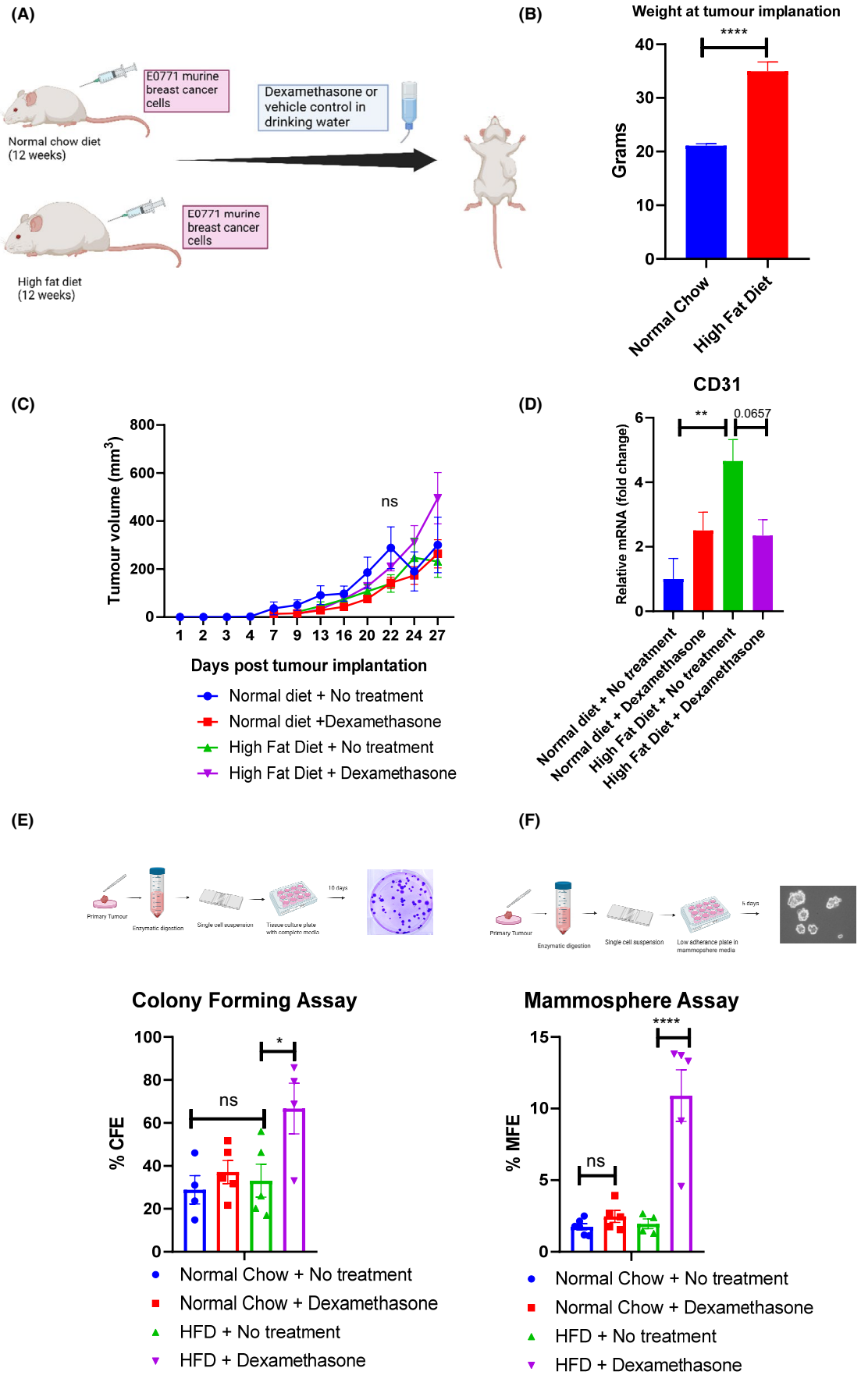
2 | MATERIALS AND METHODS

Female 8-week-old C57BL/6N mice (Charles Rivers) were fed a normal chow diet (Lab Supply, Advanced Protocol PicoLab Verified 75) or high-fat diet (Datesand, Mouse Diet High Fat Pellets) for 12 weeks. E0771 cells (CH3 BioSystems) were authenticated by short tandem repeat profiling, screened for pathogens by the manufacturer, and maintained in DMEM (Sigma) with 10% fetal bovine serum (Sigma) and 20 mM HEPES (Invitrogen) at 37°C in an atmosphere of 95% air/5% CO_2. 1×10^6 E0771 murine breast cancer cells were resuspended in 100 µl Matrigel:PBS (1:1) and injected into the 4th mammary fat pad. Mice were randomly assigned to treatment groups (Figure 1A). In vivo dexamethasone treatment (water-soluble dexamethasone; D2915, Sigma) was commenced when tumors were ~150 mm^3 (21 days post tumor implantation) at a clinically relevant dose of 0.1 mg/kg in drinking water for 5 out of 7 consecutive days for a total of 3 weeks.[5] Treatment groups of mice were normal chow diet ($n = 8$), normal chow diet + dexamethasone ($n = 7$), high-fat diet ($n = 6$), high-fat diet/dexamethasone ($n = 5$). Tumor volumes were measured and calculated three times weekly and excised when the tumor reached a mean diameter of 12 mm. For all in vivo experiments, mice were housed in individually ventilated cages according to EU Directive 2010/63 at constant temperature and humidity with a 12-h light/dark cycle and fed standard chow. The welfare of all the mice was monitored daily and health screening was carried out regularly as per the policy of the licensed establishment. Two mice were removed from the study in the normal chow diet-only treatment group due to no tumor uptake. No mortality was observed in any treatment group and mice were euthanized using exposure to CO_2. No adverse events were noted for in vivo experiments. The experimental protocols were compliant with the ARRIVE guidelines and Individual License Number I181 under the Project License Number P045. Tumors were dissociated and ex vivo analysis of mammospheres, colony-forming ability, and aldehyde dehydrogenase (ALDH) was determined as previously described.[11–13] RNA was isolated using the RNAeasy kit (Qiagen) and gene expression was quantified by real-time PCR with primers listed in Table S1.

3 | RESULTS

Female mice fed a high-fat diet (HFD) for 12 weeks were significantly heavier than mice fed a normal diet (Figure 1B; $p < .0001$). Contrary to the previous reports of diet-induced obesity-promoting E0771 tumor growth[14,15] we found no difference in the tumor growth rate between any of the groups; normal diet and no treatment ($n = 8$), normal diet and dexamethasone ($n = 7$), HFD, and no treatment ($n = 6$), HFD and dexamethasone ($n = 5$) (Figure 1C). GCs were previously reported to have anti-angiogenic activity in solid tumors[16] and, therefore, we measured the gene expression of the endothelial marker *Cd31* (also known as *Pecam-1*). There was no difference in *Cd31* gene expression between mice on a normal diet treated with or without dexamethasone (Figure 1D). However, mice fed a HFD had a significantly increased expression (Figure 1D, $p = .0073$), in line with previous reports of obesity inducing angiogenesis in breast cancer,[15] and this was partially inhibited by dexamethasone treatment although it did not reach significance (Figure 1D, $p = .0657$). Ex vivo analysis of the dissociated tumor cells found there was no increase in colony-forming ability in the mice on a normal diet treated with dexamethasone or mice fed a HFD (Figure 1E). However, there was a significant increase in colony-forming ability, indicative of enhanced clonogenicity, in the mice fed a HFD and treated with dexamethasone (Figure 1E, $p = .0432$). In addition, mammosphere forming efficiency was analyzed in the dissociated tumor cells. Similarly, there was no increase in mammospheres in mice on a normal diet treated with dexamethasone or those on a HFD (Figure 1F). However, there was an approximately 10-fold increase in mammospheres in mice on a HFD treated with dexamethasone, compared to the other treatment groups, indicative of enhanced stemness (Figure 1E, $p = .0001$). Together these results indicate that tumor cells from obese mice treated with dexamethasone have increased self-renewal activity and cell survival compared to mice on a normal diet treated with dexamethasone. ALDH has been extensively studied in breast cancer as a marker for cancer stem cells. In addition, it is associated with chemoresistance and poor survival in patients.[17] Mice on a normal diet, or a HFD, did not have an increased $ALDH^+$ bright population (Figure 2A). However, in line with previous results mice fed a HFD and treated with dexamethasone had an increased $ALDH^+$ bright subpopulation compared to the other treatment groups (Figure 2A; $p = .0302$). Next, we measured the pluripotency transcription factors *Nanog*,

FIGURE 1 High fat diet and dexamethasone does not alter tumor growth but promotes ex vivo mammosphere and colony formation. (A) Overview of experimental design. Female C57BL/6N mice were fed a normal chow diet ($n = 17$) or high fat diet (HFD) for 12 weeks ($n = 11$). E0771 murine tumor cells were implanted into the mammary fat pad and mice were randomly allocated no treatment or dexamethasone (0.1 mg/kg) in the drinking water for 5 out of 7 days for 3 weeks. Tumors were excised for ex vivo analysis. (B) Weight of mice following 12 weeks of normal chow diet or HFD. (C) Tumor growth rate of mice in the following treatment groups; normal chow diet and no treatment ($n = 10$), normal chow diet and dexamethasone ($n = 7$), HFD and no treatment ($n = 6$), HFD and dexamethasone ($n = 5$). (D) Expression of mRNA *Cd31* within excised E0771 tumors following normal diet or HFD and treatment with dexamethasone. (E) Ex vivo colony-forming ability and (F) Mammosphere formation following different diets or treatment with dexamethasone. Data points are mean ± SEM. $n \geq 3$. $^*p < .05$; $^{**}p < .01$ (one-way ANOVA or two-tailed Student *t*-test)


(A)
E0771 murine breast cancer cells
Normal chow diet (12 weeks)
Dexamethasone or vehicle control in drinking water
E0771 murine breast cancer cells
High fat diet (12 weeks)
(B)
Weight at tumour implanation
****
Grams
Normal Chow
High Fat Diet
(C)
Tumour volume (mm3)
ns
Days post tumour implantation
Normal diet + No treatment
Normal diet +Dexamethasone
High Fat Diet + No treatment
High Fat Diet + Dexamethasone
(D)
CD31
**
0.0657
Relative mRNA (fold change)
Normal diet + No treatment
Normal diet + Dexamethasone
High Fat Diet + No treatment
High Fat Diet + Dexamethasone
(E)
Primary Tumour
Enzymatic digestion
Single cell suspension
Tissue culture plate with complete media
10 days
(F)
Primary Tumour
Enzymatic digestion
Single cell suspension
Low adherance plate in mammosphere media
5 days
Colony Forming Assay
% CFE
ns
*
Normal Chow + No treatment
Normal Chow + Dexamethasone
HFD + No treatment
HFD + Dexamethasone
Mammosphere Assay
% MFE
****
ns
Normal Chow + No treatment
Normal Chow + Dexamethasone
HFD + No treatment
HFD + Dexamethasone

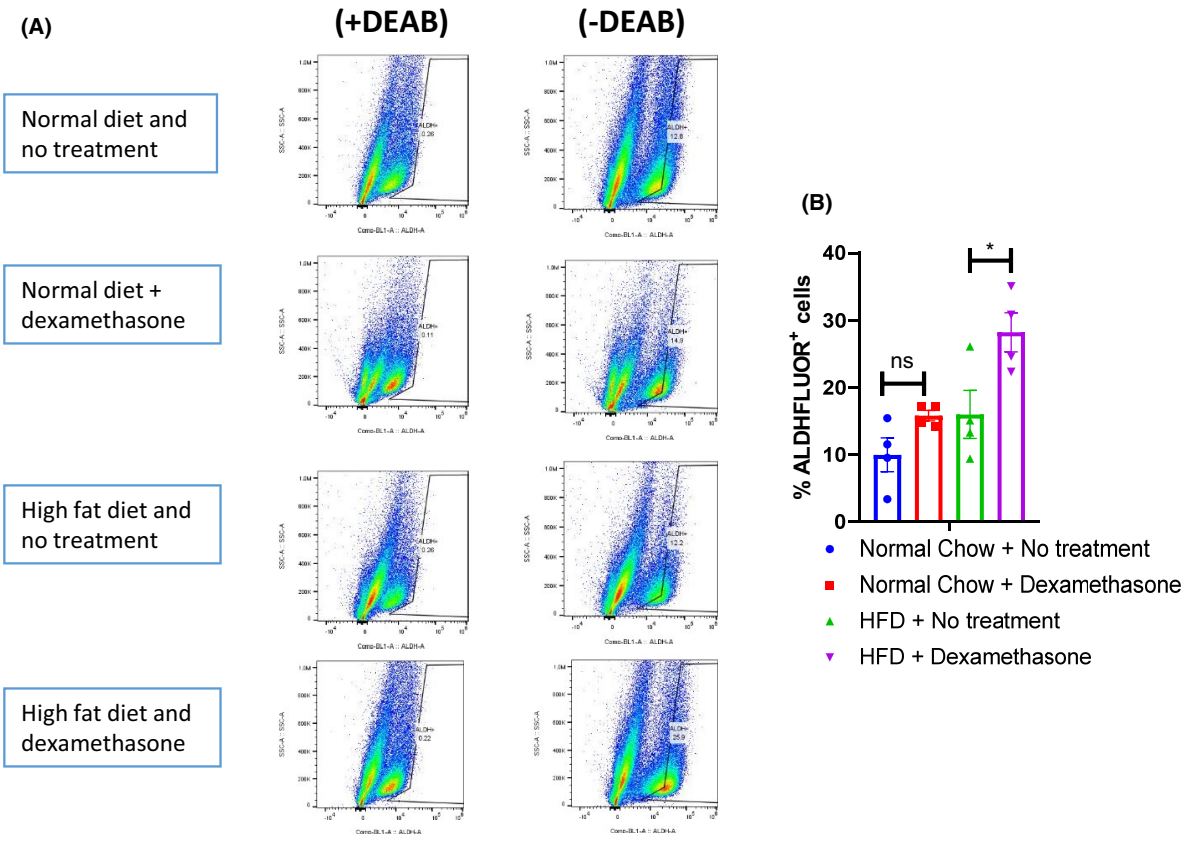

(A)
(+DEAB)
(-DEAB)
Normal diet and no treatment
Normal diet + dexamethasone
High fat diet and no treatment
High fat diet and dexamethasone
(B)
% ALDHFLUOR+ cells
ns
*
Normal Chow + No treatment
Normal Chow + Dexamethasone
HFD + No treatment
HFD + Dexamethasone
(C)
Relative mRNA (fold change)
SOX2
NANOG
Normal diet + No treatment
Normal diet + Dexamethasone
High Fat Diet + No treatment
High Fat Diet + Dexamethasone
(D)
Relative mRNA (fold change)
0.0506
ROR1
WNT5A
(E)
NR3CL1
FKBP51
ns

FIGURE 2 High fat diet and dexamethasone promotes an ALDH$^+$ bright subpopulation and pluripotency transcription factors. (A, B) Ex vivo analysis of an ALDH bright subpopulation of cells in excised E0771 tumors from mice fed either a normal or high fat diet and/or treated with dexamethasone (0.1 mg/kg) in the drinking water for 5 out of 7 days for 3 weeks. Expression of mRNA (C) *Sox2* and *Nanog*, (D) *Ror1* and *Wnt5a* and (E) *Nr3cl1* and *Fkbp51* from excised E0771 tumors following normal diet or HFD and treatment with dexamethasone. Data points are mean ± SEM. $n \geq 3$. $^*p < .05$; $^{**}p < .01$ (one-way ANOVA or two-tailed Student's *t*-test). SSC, side scatter

Oct4 (also known as *Pou5F1*), and *Sox2*. *Oct4* expression was not detectable in the tumors of mice from any treatment group. *Sox2* gene expression was induced ~6-fold by both dexamethasone in the mice fed a normal diet and in the mice fed a HFD and this increased to 10-fold in mice fed a HFD and treated with dexamethasone (Figure 2B; normal diet vs. normal diet + dexamethasone $p = .0003$; normal diet vs. HFD $p = .0003$; HFD vs. HFD + dexamethasone $p = .0025$). Furthermore, HFD mice treated with dexamethasone also had a ~10-fold increase in *Nanog* expression, compared to the other treatment groups (Figure 2B; $p < .0001$). It has been reported that GCs increase stemness and metastasis through activation of the non-canonical Wnt signaling axis[4,5] and therefore we investigated if *Ror1*, and its ligand *Wnt5a*, was also activated in our tumors. Treatment with dexamethasone on a normal diet increased expression of *Wnt5A* (Figure 2C; $p = .0506$), but this was not increased by HFD (Figure 2C). *Ror1* expression was not altered in any of the treatment groups (Figure 2C). In addition, genes classically regulated by GCs, *Nrcl1*, and *Fkbp51*,[18] were not induced in the tumors of any group (Figure 2D). Together, this indicates that dexamethasone promotes a cancer stem cell phenotype in obesity through upregulation of pluripotency transcription factor via non-classical GC signaling.

4 | DISCUSSION

Adjuvant GC drugs (such as dexamethasone) are routinely used during the treatment of cancer to mitigate the undesirable effects of chemotherapy such as nausea and fatigue and to stimulate appetite.[19] In addition, they reduce hypersensitivity reactions to some chemotherapy agents and enhance tolerability to allow higher chemotherapy doses and more frequent cycles.[19] However, studies have revealed that GCs can promote cancer progression, nevertheless, the literature remains conflicting, and it is not clear how GCs either promote or inhibit tumor progression in different cancer types and by different mechanisms. Here, for the first time, we describe that obesity also has a profound effect on the ability of dexamethasone to promote the cancer stem cell phenotype, that does not occur in lean mice. Interestingly, overall, we found that obesity or dexamethasone alone had a limited effect on promoting cancer stem cells (Figure 1F, Figure 2A). This is in contrast to previous reports demonstrating that both obesity and dexamethasone alone promote metastasis via stem cell-related signaling.[3,4,14,20–23] In our study, we found that dexamethasone promoted expression of the pluripotency transcription factor *Sox2* in both normal diet and obese mice (Figure 2B), potentially indicating that a longer treatment time may promote stemness in lean mice. On the contrary, several studies have shown that dexamethasone decreases *Sox2* and thereby inhibits cancer stem cells.[24,25] In addition, similar to our study, dexamethasone did not promote *Ror1* expression in pancreatic cells[24] (Figure 2C). Together these results indicate that GCs are a regulator of *Sox2* expression, however, its activation or inhibition is dependent upon the cell type and/or microenvironment. Indeed, a recent preprint on BioRxiv demonstrates, through single-cell transcriptomics, that following acute GC exposure, cerebral organoids elicit differential effects depending on cell type.[26] Similar experiments utilizing single-cell sequencing will aid our understanding of the cell type-specific effects of GCs within the tumor microenvironment. Patients living with obesity make up a substantial proportion of individuals with breast cancer, however, they may not benefit equitably from established therapies. It is well-described that there is a reduced efficacy of cancer treatment among obese patients, particularly for chemotherapy in which the dose is often based on ideal body weight rather than actual body weight because of toxicity concerns.[27] We now provide initial evidence that GCs may have an altered mechanism of action in obesity resulting in expansion of breast cancer stem cells, which can promote recurrence and metastasis. Initially, we hypothesized that obesity-induced activation of classical GC signaling which may promote stemness. However, known GR transcription targets *Nr3cl1* (glucocorticoid receptor) and *Fkbp51* were not altered in the tumors following dexamethasone treatment (Figure 2D). This indicates that dexamethasone use in obesity may promote stemness via a non-genomic signaling mechanism. This may involve either membrane or cytoplasmic GR that does not require nuclear translation and subsequent GR-mediated transcription. GR interacts with several kinases such as JNK, Src, PI3K,[19] and this may play an important role in the GC regulation of stemness, however, the non-genomic activities of GCs are not well understood. In summary, we provide pre-clinical evidence that administration of dexamethasone in obesity results in an increased cancer stem cell-like subpopulation in mice, and GCs may promote tumor reoccurrence in breast cancer patients also living with obesity.

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DISCLOSURE

No conflicts of interest.

AUTHORS CONTRIBUTIONS

The authors were involved in the following tasks: SA (conception and design of the study, data acquisition, data interpretation, statistical analysis of the manuscript, data interpretation, writing, and

final approval of the manuscript); OF; (data acquisition, statistical analysis of data, data interpretation, and final approval of the manuscript); DM (data acquisition); TR (conception and final approval of the manuscript).

ETHICS STATEMENT

All in vivo experiments were completed under HPRA project license P045, Individual license number I181 and complied with ARRIVE guidelines.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study is available on request.

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REFERENCES

1. Petrelli F, Bukovec R, Perego G, et al. Association of steroid use with survival in solid tumours. *Eur J Cancer*. 2020;141:105-114. doi:10.1016/j.ejca.2020.09.020
2. Sorrentino G, Ruggeri N, Zannini A, et al. Glucocorticoid receptor signalling activates YAP in breast cancer. *Nat Commun*. 2017;8:1-14. doi:10.1038/ncomms14073
3. Kostopoulou ON, Mohammad AA, Bartek J, et al. Glucocorticoids promote a glioma stem cell-like phenotype and resistance to chemotherapy in human glioblastoma primary cells: biological and prognostic significance. *Int J Cancer*. 2018;142:1266-1276. doi:10.1002/ijc.31132
4. Karvonen H, Arjama M, Kaleva L, et al. Glucocorticoids induce differentiation and chemoresistance in ovarian cancer by promoting ROR1-mediated stemness. *Cell Death Dis*. 2020;11. doi:10.1038/s41419-020-03009-4
5. Obradović MMS, Hamelin B, Manevski N, et al. Glucocorticoids promote breast cancer metastasis. *Nature*. 2019;567:540-544.
6. Obesity and overweight. Accessed May 27, 2021. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight
7. Barone I, Giordano C, Bonofiglio D, Andò S, Catalano S. The weight of obesity in breast cancer progression and metastasis: clinical and molecular perspectives. *Semin Cancer Biol*. 2020;60:274-284.
8. Kroenke CH, Chen WY, Rosner B, Holmes MD. Weight, weight gain, and survival after breast cancer diagnosis. *J Clin Oncol*. 2005;23:1370-1378. doi:10.1200/JCO.2005.01.079
9. Wester VL, Staufenbiel SM, Veldhorst MAB, et al. Long-term cortisol levels measured in scalp hair of obese patients. *Obesity*. 2014;22:1956-1958. doi:10.1002/oby.20795
10. Lee MJ, Pramyothin P, Karastergiou K, Fried SK. Deconstructing the roles of glucocorticoids in adipose tissue biology and the development of central obesity. *Biochim Biophys Acta - Mol Basis Dis*. 2014;1842:473-481.
11. Annett S, Moore G, Short A, et al. FKBPL-based peptide, ALM201, targets angiogenesis and cancer stem cells in ovarian cancer. *Br J Cancer*. 2020;122(3):361-371. doi:10.1038/s41416-019-0649-5
12. McClements L, Annett S, Yakkundi A, et al. FKBPL and its peptide derivatives inhibit endocrine therapy resistant cancer stem cells and breast cancer metastasis by downregulating DLL4 and Notch4. *BMC Cancer*. 2019;19:351. doi:10.1186/s12885-019-5500-0
13. McClements L, Yakkundi A, Papaspyropoulos A, et al. Targeting treatment-resistant breast cancer stem cells with FKBPL and its peptide derivative, AD-01, via the CD44 pathway. *Clin Cancer Res*. 2013;19:3881-3893. doi:10.1158/1078-0432.CCR-13-0595
14. Bousquenaud M, Fico F, Solinas G, Rüegg C, Santamaria-Martínez A. Obesity promotes the expansion of metastasis-initiating cells in breast cancer. *Breast Cancer Res*. 2018;20:1-11. doi:10.1186/s13058-018-1029-4
15. Kolb R, Kluz P, Tan ZW, et al. Obesity-associated inflammation promotes angiogenesis and breast cancer via angiopoietin-like 4. *Oncogene*. 2019;38:2351-2363. doi:10.1038/s41388-018-0592-6
16. Liu B, Goodwin JE. The effect of glucocorticoids on angiogenesis in the treatment of solid tumors. *J Cell Signal*. 2020;1:42. doi:10.33696/signaling.1.011
17. Clark DW, Palle K. Aldehyde dehydrogenases in cancer stem cells: potential as therapeutic targets. *Ann Transl Med*. 2016;4(24):518. doi:10.21037/atm.2016.11.82
18. Annett S, Moore G, Robson T. FK506 binding proteins and inflammation related signalling pathways; basic biology, current status and future prospects for pharmacological intervention. *Pharmacol Ther*. 2020;215:107623. doi:10.1016/j.pharmthera.2020.107623
19. Lin KT, Wang LH. New dimension of glucocorticoids in cancer treatment. *Steroids*. 2016;111:84-88.
20. Hillers-Ziemer LE, McMahon RQ, Hietpas M, et al. Obesity promotes cooperation of cancer stem-like cells and macrophages to enhance mammary tumor angiogenesis. *Cancers*. 2020;12(2):502. doi:10.3390/cancers12020502
21. Sorrentino G, Ruggeri N, Zannini A, et al. Glucocorticoid receptor signalling activates YAP in breast cancer. *Nat Commun*. 2017;8:14073. doi:10.1038/ncomms14073.
22. Liu LI, Aleksandrowicz E, Schönsiegel F, et al. Dexamethasone mediates pancreatic cancer progression by glucocorticoid receptor, TGFβ and JNK/AP-1. *Cell Death Dis*. 2017;8:e3064. doi:10.1038/cddis.2017.455
23. Annett S, Moore G, Robson T. Obesity and cancer metastasis: molecular and translational perspectives. *Cancers*. 2020;12:3798. doi:10.3390/cancers12123798
24. Suzuki S, Okada M, Sanomachi T, et al. Therapeutic targeting of pancreatic cancer stem cells by dexamethasone modulation of the MKP-1–JNK axis. *J Biol Chem*. 2020;295:18328-18342. doi:10.1074/jbc.RA120.015223
25. Jiang Z, Zhang C, Liu X, et al. Dexamethasone inhibits stemness maintenance and enhances chemosensitivity of hepatocellular carcinoma stem cells by inducing deSUMOylation of HIF-1α and Oct4. *Int J Oncol*. 2020;57:780-790. doi:10.3892/ijo.2020.5097
26. Cruceanu C, Dony L, Krontira AC, et al. Cell-type specific impact of glucocorticoid receptor activation on the developing brain. *bioRxiv*. doi:10.1101/2020.01.09.897868
27. Ross KH, Gogineni K, Subhedar PD, Lin JY, McCullough LE. Obesity and cancer treatment efficacy: existing challenges and opportunities. *Cancer*. 2019;125:1588-1592.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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