

SHORT REPORT

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Insulin and IGF-1, but not 17 β -estradiol, alter the subcellular localization of MIER1 α in MCF7 breast carcinoma cells

Shengnan Li, Gary D. Paterno and Laura L. Gillespie*

Abstract

Background: MIER1 α is a transcriptional regulator that interacts with estrogen receptor α and inhibits estrogen-stimulated growth of breast carcinoma cells. Interestingly, analysis of MIER1 α subcellular localization in breast samples revealed a stepwise shift from the nucleus to the cytoplasm during progression to invasive carcinoma. Previously, we demonstrated that MIER1 α is nuclear in MCF7 cells yet it does not contain a nuclear localization signal. Instead MIER1 α is targeted to the nucleus through interaction and co-transport with HDAC 1 and 2.

Results: In this study, we demonstrate that treatment of MCF7 breast carcinoma cells with either insulin or insulin-like growth factor affects the subcellular localization of MIER1 α . Both factors reduce the percentage of cells with nuclear MIER1 α from 81 and 89 to 41 and 56 %, respectively. Treatment with 17 β -estradiol, on the other hand, had no effect and MIER1 α remained nuclear.

Conclusions: Our data demonstrate that insulin and IGF-1 can contribute to loss of nuclear MIER1 α in the MCF7 breast carcinoma cell line.

Keywords: MIER1 α , Transcriptional regulator, Breast cancer, MCF7, Insulin, IGF-1, Estrogen, Subcellular localization

Background

MIER1 α is a transcriptional repressor [1, 2] that has been implicated as a tumour suppressor in breast cancer [3]. It interacts with ER α and inhibits estrogen-stimulated anchorage-dependent growth of breast carcinoma cells [3]. Moreover, analysis of patient breast biopsies revealed a dramatic reduction in nuclear MIER1 α during progression, from 75 % nuclear MIER1 α in normal samples to 51 % nuclear in ductal carcinoma in situ to 4 % nuclear in invasive ductal carcinoma [3]. Thus loss of nuclear MIER1 α is associated with breast cancer progression.

MIER1 represses transcription through several distinct mechanisms: it can recruit histone deacetylase (HDAC) 1 and 2 to the promoter of responsive genes [1]; it can bind Creb binding protein (CBP) and inhibit its histone acetyltransferase activity [4]; finally, it can interact directly with

transcription factors such as Sp1 and displace them from their cognate site on target gene promoters [2]. All of these functions are dependent on localization of MIER1 α in the nucleus, yet it does not contain a functional NLS [5]. Instead, translocation into the nucleus is dependent on interaction and co-transport with HDAC1 and 2 [6]. In this report, we show that localization of MIER1 α in the nucleus of MCF7 cells is significantly reduced by treatment with insulin or IGF-1, but not by 17 β -estradiol (E2). This suggests that insulin or IGF-1 could attenuate MIER1 α 's transcriptional repressor/chromatin modifying functions in MCF7.

Methods

The MCF7 breast carcinoma cell line was obtained from the ATCC and cultured in DMEM (GIBCO) containing 10 % serum [7.5 % calf serum (CS) + 2.5 % fetal bovine serum (FBS)] (GIBCO), in a humidified 37 °C incubator with 5 % CO₂. Insulin was purchased from Life Technologies and used at a concentration of 10 μ g/ml. IGF-1

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was purchased from PeproTech and used at a concentration of 10 ng/ml. 17 β -estradiol was purchased from Sigma-Aldrich and used at a concentration of 10⁻⁸ M. For experiments using 17 β -estradiol, cells were cultured in phenol red-free DMEM (GIBCO) supplemented with 10 % charcoal-stripped FBS (Hyclone). Cells were treated for 4 h with insulin, IGF-1 or 17 β -estradiol prior to fixation. Construction of the human *mier1 α* sequence (GenBank: AY124188) in the CS3 + MT vector has been described previously [1]. Transient transfection, confocal microscopy, antibodies used and Z-stack analysis were performed as described in [6, 7]. Subcellular localization was scored as 'nuclear' if the nucleus was intensely stained, with little or no cytoplasmic staining; 'cytoplasmic' if staining was primarily in the cytoplasm, with little or no staining in the nucleus; 'whole cell' if both the nucleus and cytoplasm were stained [6]. Statistical analysis was performed using a two-sided Fisher's exact test.

Results and discussion

Insulin alters nuclear localization of MIER1 α in MCF7 cells

We have shown previously that MIER1 α is targeted to the nucleus in MCF7 cells despite the lack of an intrinsic NLS [6, 7]. In those studies, cells were cultured in DMEM containing 10 % CS/FBS. Several laboratories, including the ATCC, add 10 μ g/ml insulin to culture media for MCF7 cells, however when we added insulin, we noticed a change in the subcellular localization pattern of MIER1 α . To investigate this effect more thoroughly, we analysed *mier1 α* -transfected MCF7 cells by confocal microscopy. In the presence of insulin, only 41 % of cells had exclusively nuclear MIER1 α (Fig. 1Ad–f, B), compared to 81 % of cells in the absence of insulin (Fig. 1Aa–c, B). The percentage cells with MIER1 α in both the nucleus and cytoplasm (whole cell staining) increased in the presence of insulin, from 18 to 42 % (Fig. 1B). Likewise, the proportion of cells with exclusively cytoplasmic MIER1 α increased over tenfold, from 1 to 17 % (Fig. 1B). These results demonstrate that in the presence of insulin, localization of MIER1 α in MCF7 cells is shifted from the nucleus to the cytoplasm.

Our previous research demonstrated that MIER1 α localizes to the nucleus through interaction and co-transport with HDAC1/2. Therefore we investigated whether insulin also affected localization of HDAC1/2. Confocal analysis demonstrated that while insulin reduces nuclear accumulation of MIER1 α (Fig. 2Ab, f, j), it does not affect localization of HDAC1 or 2 (Fig. 2Ac, g, k) and both were 100 % nuclear (Fig. 2B).

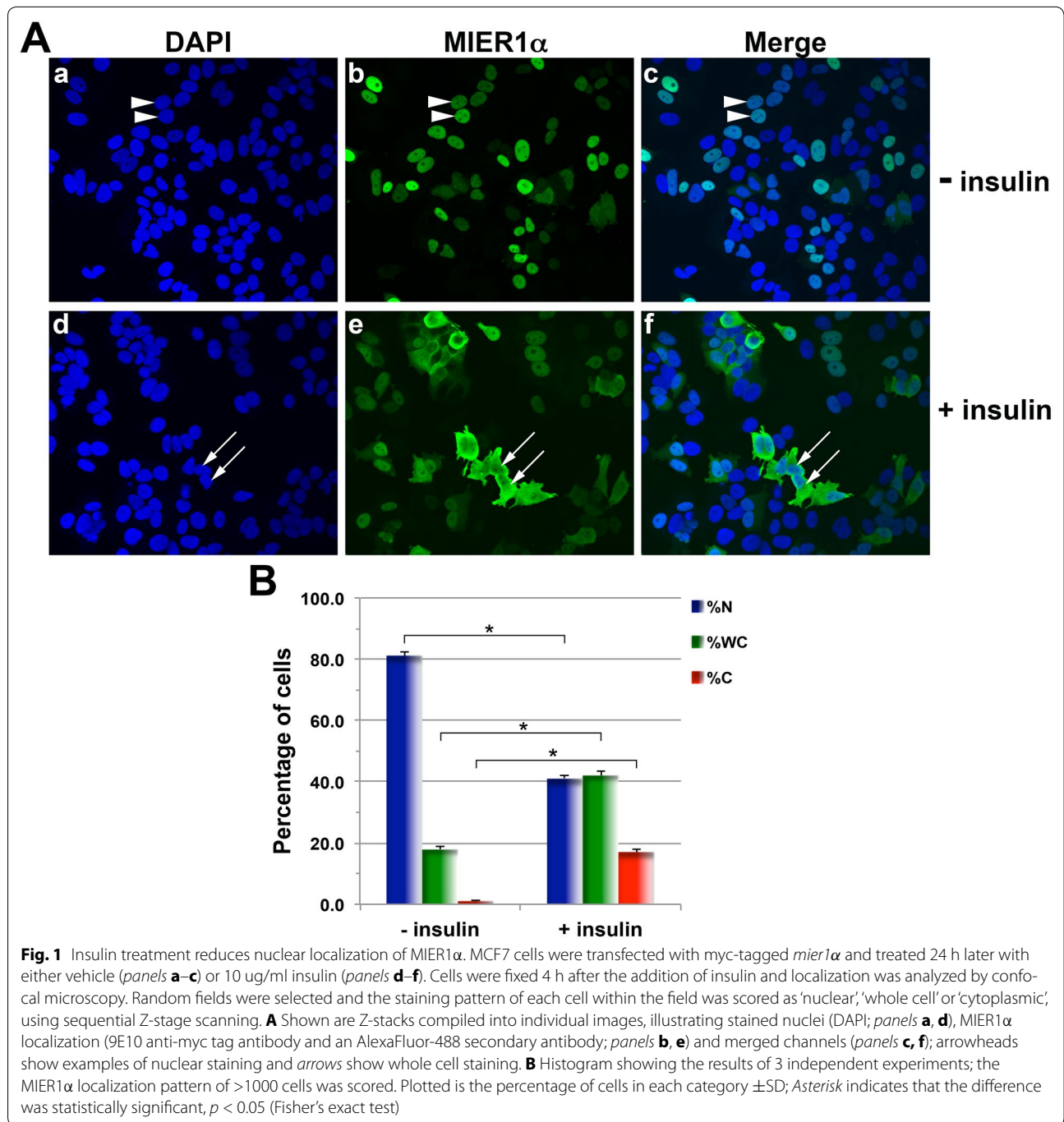
Nuclear accumulation of MIER1 α is affected by IGF-1, but not by E2

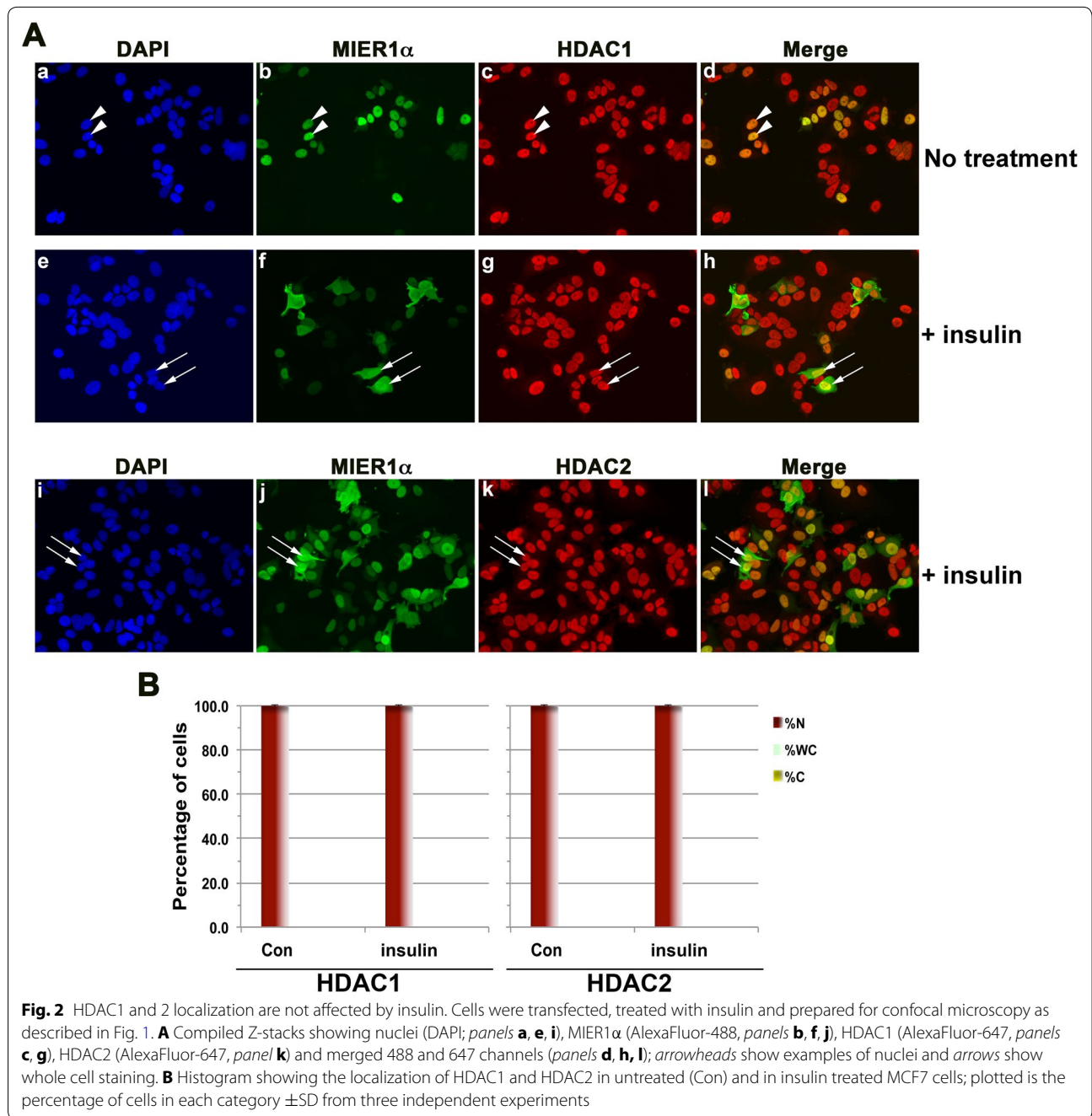
IGF-1 is closely related to insulin and both can interact with the insulin and IGF receptors, albeit with differing affinities [8]. In addition, there is a wealth of evidence implicating IGF-1 in breast cancer development and progression (reviewed in [9]) and it has been shown to increase invasiveness of MCF7 cells [10]. Since MCF7 cells express receptors for both insulin and IGFs [11], we explored the possibility that IGF-1 also affects localization of MIER1 α . As expected, confocal analysis demonstrated that IGF-1 had a similar effect on nuclear accumulation of MIER1 α (Fig. 3Ab, f, j, B). IGF-1 reduced the percentage of cells with nuclear MIER1 α from 89 to 56 % and increased the percentage with 'whole cell' staining from 10 to 40 %. The percent with 'cytoplasmic' MIER1 α was also increased from 0.3 to 4 %. Thus, both insulin and IGF-1 have similar effects on the subcellular localization of MIER1 α in MCF7.

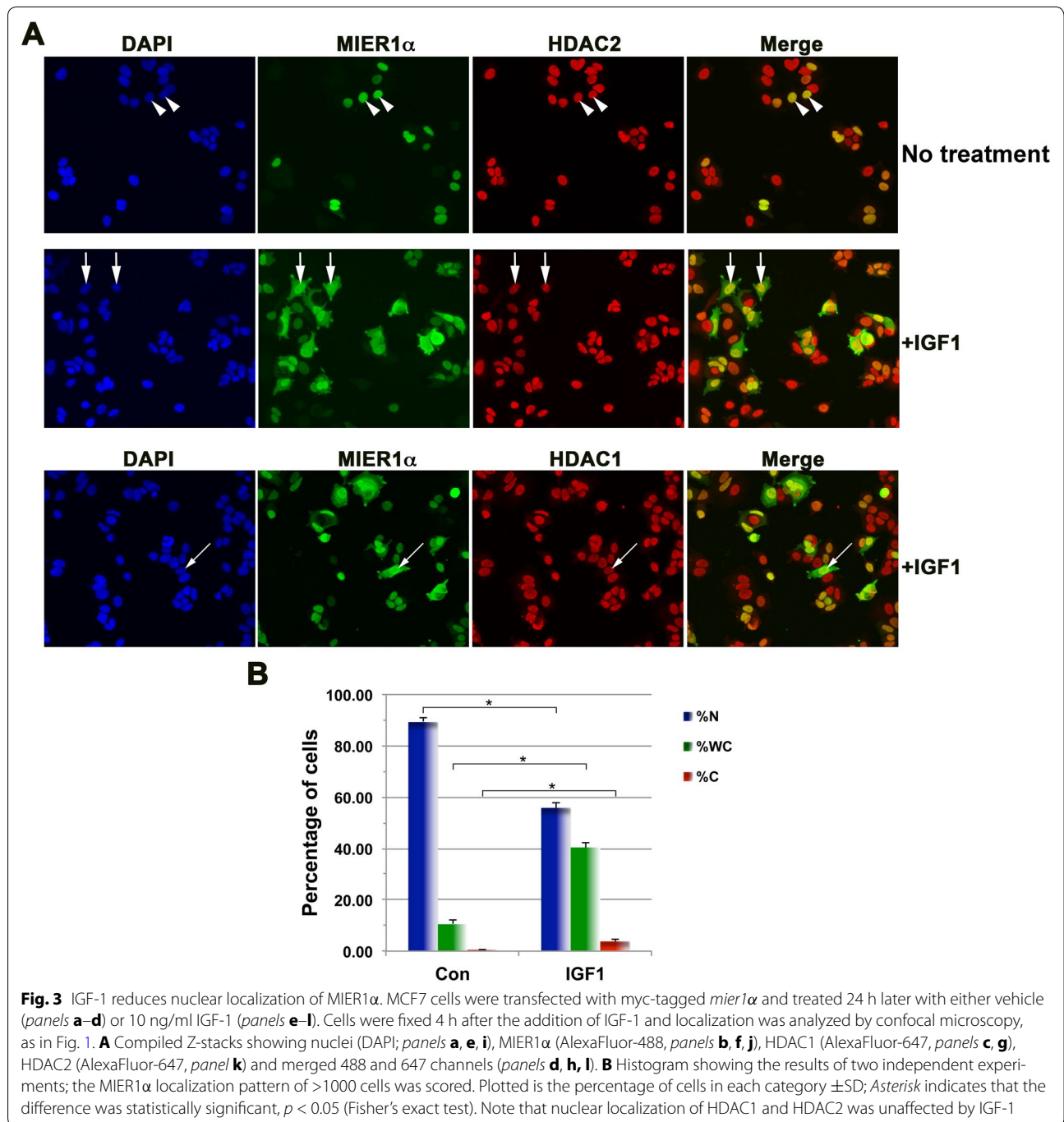
Insulin and IGFs are potent mitogens for MCF7 cells [12], leading to the question of whether changes in nuclear accumulation of MIER1 α are related to the fact that the cells are proliferating. We therefore examined MIER1 α localization in cells treated with E2, a classic mitogen for ER + breast carcinoma cells. Unlike insulin and IGF-1, E2 had no significant effect on nuclear accumulation of MIER1 α (Fig. 4). In the presence of E2, 77 % of cells displayed nuclear MIER1 α (Fig. 4Af, j, B) compared to 80 % of untreated cells (Fig. 4Ab, B). Likewise there was no significant difference in the percentage of cells with 'whole cell' or 'cytoplasmic' staining (Fig. 4B). These results demonstrate that the shift in MIER1 α localization directed by insulin and IGF-1 is not the non-specific result of cell proliferation.

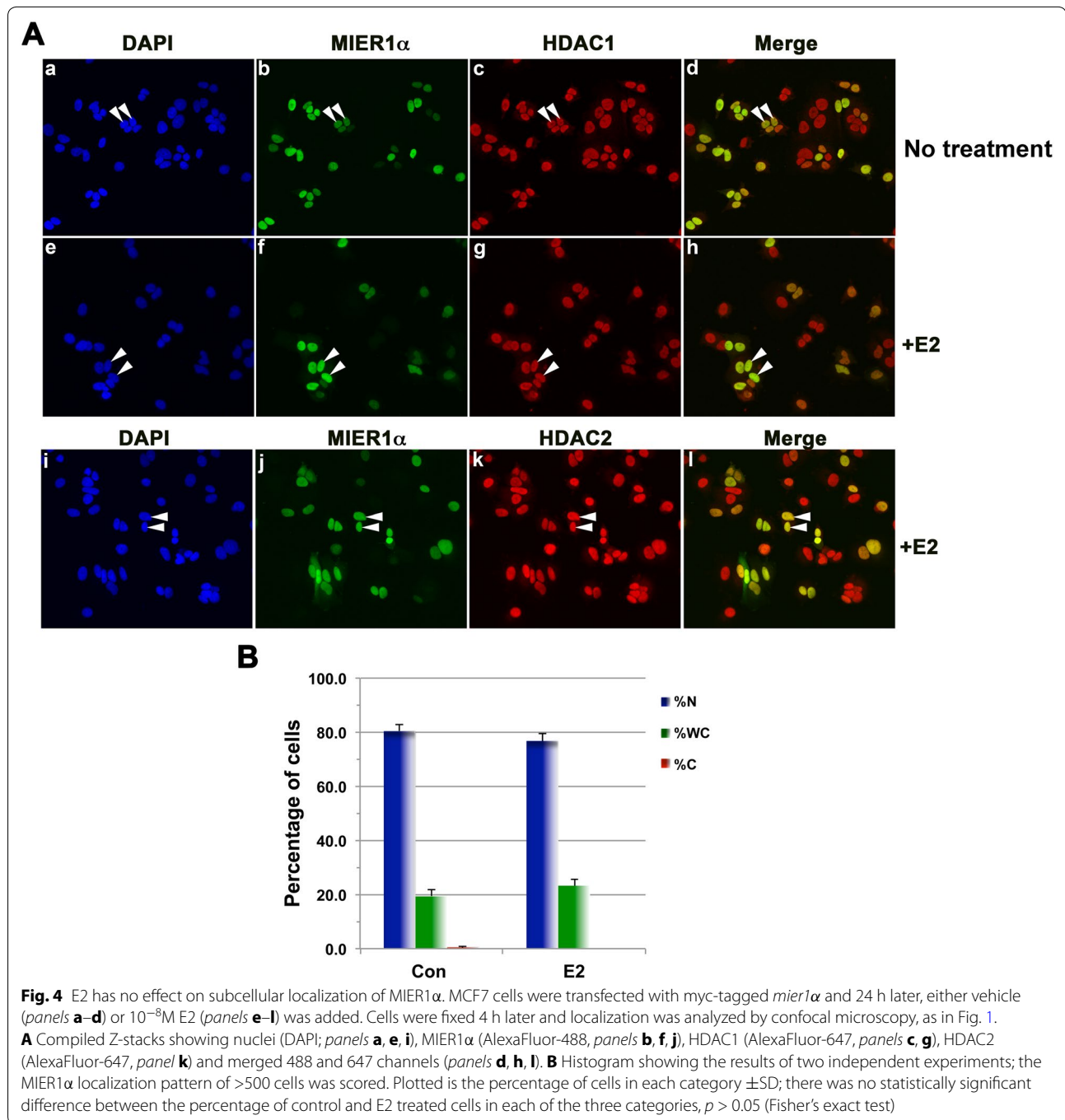
More than likely activation of one of the insulin/IGF signalling pathways is responsible for the altered MIER1 α localization in MCF7. This type of effect has been observed for the FOXO family of transcription factors. For example, FOXO is driven out of the nucleus by insulin as well as other growth factors [13] and by Src signalling [14]. In *C. elegans*, activation of DAF-2, the nematode ortholog of the IGF-1 receptor, prevents nuclear accumulation of the DAF-16 (FOXO) transcription factor [15].

The data presented here demonstrate that for MCF7 the culture conditions, specifically the common practice of including insulin in the medium, can have important consequences for studies of nuclear proteins like MIER1 α .









Abbreviations

CBP: creb binding protein; CS: calf serum; E2: 17- β estradiol; ER α : estrogen receptor alpha; FBS: fetal bovine serum; HDAC: histone deacetylase; IGF-1: insulin-like growth factor 1; NLS: nuclear localization signal.

Authors' contributions

SL performed the experiments. LLG analysed the data, prepared the Figures and wrote the manuscript. LLG and GDP participated in the design of the experiments and interpretation of the data. All authors were involved in the revisions. All authors read and approved the final manuscript.

Acknowledgements

This work was supported by a grant from the Canadian Institutes of Health Research and the Canadian Breast Cancer Foundation-Atlantic Chapter to LLG and GDP.

Compliance with ethical guidelines

Competing interests

The authors declare that they have no competing interests.

Received: 28 November 2014 Accepted: 12 August 2015

Published online: 18 August 2015

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