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Autophagy induction impairs Wnt/β-catenin signalling through β-catenin relocalisation in glioblastoma cells

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ABSTRACT

Autophagy is an evolutionary conserved process mediating lysosomal degradation of cytoplasmic material. Its involvement in cancer progression is highly controversial, due to its dual role in both limiting tumoural transformation and in protecting established tumoral cells from unfavorable conditions. Little is known about the cross-talk between autophagy and intracellular signalling pathways, as well as about autophagy impact on signalling molecules turnover.

An aberrantly activated Wnt/β-catenin signalling is responsible for tumour proliferation, invasion, and stemness maintenance. Here we show that autophagy negatively regulates Wnt/β-catenin signalling in glioblastoma multiforme (GBM) cells, through Dishevelled degradation. We also provide the first evidence that autophagy promotes β-catenin relocalisation within the cell, by inducing a decrease of the nuclear protein fraction. In particular, upon autophagy induction, β-catenin appears mainly localized in sub-membrane areas where it associates with N-cadherin to form epithelial-like cell-cell adhesion structures.

Our data indicate, for the first time, that autophagy induction results in Wnt signalling attenuation and in β-catenin relocalisation within the GBM cell. These findings further support the idea that autophagy modulation could represent a potential therapeutical strategy to contrast GBM progression.

1. Introduction

Macroautophagy (hereafter referred as autophagy) is an evolutionary conserved and finely regulated process, mediating lysosomal degradation of cytoplasmic material, long-lived and damaged proteins, and entire organelles [1,2]. During autophagy, double-membrane vesicles, named autophagosomes, form and engulf cytoplasmic material, which is then delivered to lysosomes for degradation [3]. In shortage of nutrients, mainly aminoacids, the nutrient sensor mTOR is inhibited and Ulk1 complex can drive a massive autophagosome formation [4]. Nevertheless, in physiological conditions, a basal level of autophagy contributes to maintain the proper cell homeostasis, both during embryogenesis and adulthood, and indeed its deregulation has been associated to many human diseases, including cancer [5–7]. We have recently demonstrated that autophagy modulation regulates the migration and the invasion capabilities of glioblastoma multiforme (GBM) cells, by down-regulating epithelial-to-mesenchymal transition (EMT) factors SNAIL and SLUG, and, subsequently, up-regulating cadherin expression [8].

β-catenin is a multitasking and evolutionary conserved protein that plays a crucial role in a plethora of developmental and homeostatic processes [9,10]. It is, in fact, a structural component of the cell-cell adhesion structures, and as a component of the canonical Wnt pathway, it regulates cellular proliferation, adhesion, and movement [9,11]. Deregulation of Wnt signalling is often associated with initiation, proliferation, and invasion of several cancers, including GBM [11,12]; β-

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catenin and other components of the Wnt pathway are, in fact, commonly overexpressed and the pathway is constitutively active in GBM [13,14]. The Wnt/β-catenin pathway plays also a role in EMT, by promoting transcription and stabilization of the EMT activators SNAIL and ZEB1 [15,16], or by other mechanisms [17,18].

Few literature evidence exist on the relationship between autophagy and Wnt/β-catenin signalling [19–21]. It has been shown that Wnt/β-catenin activity represses autophagy and the expression of the autophagic adaptor p62, and indeed that nutrient deprivation induces β-catenin degradation via autophagic machinery [22]. In 2014, Gao et al. demonstrated that, in colorectal cancer cells, autophagy negatively regulates Wnt signalling by promoting the degradation of the pathway mediator Dishevelled [23]. Moreover, it has been recently shown that the anti-helminthic drug Niclosamide induces autophagy and downregulates the Wnt and other pro-survival signal transuction pathways in GBM cells [24].

Our findings indicate, for the first time, that autophagy induction attenuates the Wnt signalling in GBM cellular models and that it leads to β-catenin relocalisation within the cell. These findings further support the idea that autophagy modulation could represent a potential therapeutical strategy to contrast GBM proliferation and invasiveness.

2. Material and methods

2.1. Cell culture

Human GL15 cells were kindly provided by Dr. E. Castigli, Perugia University, Italy. Primary human GBM130 cells were obtained, as previously described [25], after surgery at Policlinico Umberto I (Rome) from GBM patients that gave a written informed consent to the research proposals. The study was approved by the Institutional Ethics Committee of Sapienza University and by Ministry of Health. Histopathological typing was done according to the WHO criteria resulting as grade IV. Human GL15, U87MG cells and GBM130 were cultured in DMEM (Lonza, Basel, Switzerland), supplemented with 10% heat-inactivated FBS (Euroclone, Milan, Italy) and 1% penicillin/streptomycin solution (Euroclone, Milan, Italy). Cells were grown at 37 °C in a 5% CO_2 humidified atmosphere. For autophagy induction, cells were cultured in Earle's Balanced Salt Solution (Sigma Aldrich, Milan, Italy) or in the presence of 250 nM Torin 1 (Sigma Aldrich, Milan, Italy) for 18 h. In order to analyse the autophagic flux, 20 μM Chloroquine(CQ) was added to the culture media.

shBeclin and sh-Ctr cells were prepared by lentiviral infection as previously described [8]. GL15 cells were infected by incubation with lentivirus containing supernatant for 6 and 8 h in presence of 4 μg/ml poly-brene.

2.2. qPCR

Total RNA was isolated with the RNeasy mini kit (QIAGEN, Hilde, Germany). 1 μg RNA was retrotranscribed using M-MLV enzyme and oligodT (Promega, Madison, Wisconsin, USA). qPCR was performed using SYBR GREEN PCR Master Mix Applied Biosystems (Thermo Fisher Scientific, Waltham, Massachusetts, USA), with the QuantStudio 12 K Flex Applied biosystem. The following pairs of primers were used: axin2 (5′-AGTGTGAGGTCCACGGAAAC-3′; 5′-CTTCACACTGCGATGCA TTT-3′), cyclin D1: (5′-CTGGCCATGAACTACCTGGA-3′; 5′-CTCCGCCT CTGGCATTTTGG-3′); c-myc: (5′-AGCGACTCTGAGGAGGAACA-3′; 5′-CTCTGACCTTTTGCCAGGAG'); β2m (5′-CTCCGTGGCCTTAGCT GTG-3′; 5′-TCTCTGCTGGATGACGTGAG-3′). mRNA expression level for the genes of interest was calculated by means of the ddCt method, with β2m expression as standard. The mRNA levels were then expressed as variations in respect to the control condition. Reported values are the means of three independent biological replicates.

2.3. Western blotting and antibodies

Proteins were separated by means of SDS-PAGE and electroblotted onto nitrocellulose (GE Healthcare, Life Sciences, Little Chalfont, Buckinghamshire, UK). Membranes were incubated with primary antibodies diluted in PBS/5% non-fat dry milk/0.1% Tween-20 overnight at 4 °C. Detection was achieved using horseradish peroxidase-conjugated secondary antibody (Bio-Rad Laboratories, Milan, Italy) and visualized with ECL plus (GE Healthcare, Life Sciences, Little Chalfont, Buckinghamshire, UK). The following primary antibodies were used: anti-p62 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-LC3B (Cell Signalling, Danvers, MA, USA), anti-BECN1 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-β-catenin (BD Biosciences, Franklin Lakes, New Jersey, USA), anti-active β-catenin (S37/T41) (Millipore, Billerica, MA, USA), anti-Dvl2 (Cell Signalling, Danvers, MA, USA), anti-Lamin A/C (Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-LDH (Santa Cruz Biotechnology, Santa Cruz, CA, USA), and anti- β-Actin (Sigma Aldrich, Milan, Italy).

2.4. Cell lysis and subcellular fractionation

The appropriate amount of Nucleus Buffer (1 mM K_2HPO_4, 14 mM $MgCl_2$, 150 mM NaCl, 1 mM EGTA supplemented with 0.1 mM DTT, 0.3% Triton and protease inhibitor cocktail) were added to pelletted cells and, after incubation on ice for 30 min, centrifuged at 850*g* for 10 min, to isolate the nuclear fraction. Nuclei were washed twice with fractionation buffer, centrifuged as above and resuspended in RIPA Buffer (50 Mm Tris HCl pH 7.4, Triton 1%, Na Deoxycholate 0.25%, SDS 0.1%, 150 mM NaCl, 1 mM EDTA, 5 mM $MgCl_2$ supplemented with protease inhibitors cocktail). Samples were then briefly sonicated (Hold 6 constant, 30″). Proteins concentration was determined using Lowry protein assay (Bio-Rad, Hercules, CA, USA).

2.5. Immunocytochemistry and confocal analysis

Cells were grown on coverslips and fixed with 4% PFA in PBS, followed by permeabilization with 0.1% Triton X-100 in PBS. β-catenin (BD Biosciences, Franklin Lakes, New Jersey, USA) and N-cadherin (Santa Cruz Biotechnology, Santa Cruz, CA, USA) primary antibodies were incubated overnight at 4 °C and visualized by means of Alexa Fluor (Invitrogen, Carlsbad, CA, USA). Coverslips were mounted in antifade (SlowFade; Invitrogen, Carlsbad, CA, USA) and examined under a confocal microscope (TCS SP8; Leica, Wetzlar, Germany), equipped with a 40 × 1.40–0.60 NA HCX Plan Apo oil BL objective at RT.

Regarding ImageJ software analysis, the pixels of two 8-bit images (red and green channels of each image) are considered colocalized if their intensities are higher than the threshold of their channels (set at 50) and if the ratio of their intensity is higher than the ratio setting value (set at 50%). Colocalization was assessed by calculating the Pearson's correlation coefficient *r* of at least 10 cells analyzed in two independent experiments. The Pearson's correlation coefficient was expressed as mean ± SD. The fluorescence intensity of each fluorochrome was simultaneously analyzed and plotted.

2.6. Statistical analysis

All experiments were performed at least three times. Sigma Plot software was used for statistical analysis. Statistical significance was determined by using the Student's *t*-test. P value $\leq .05$ was considered significant.

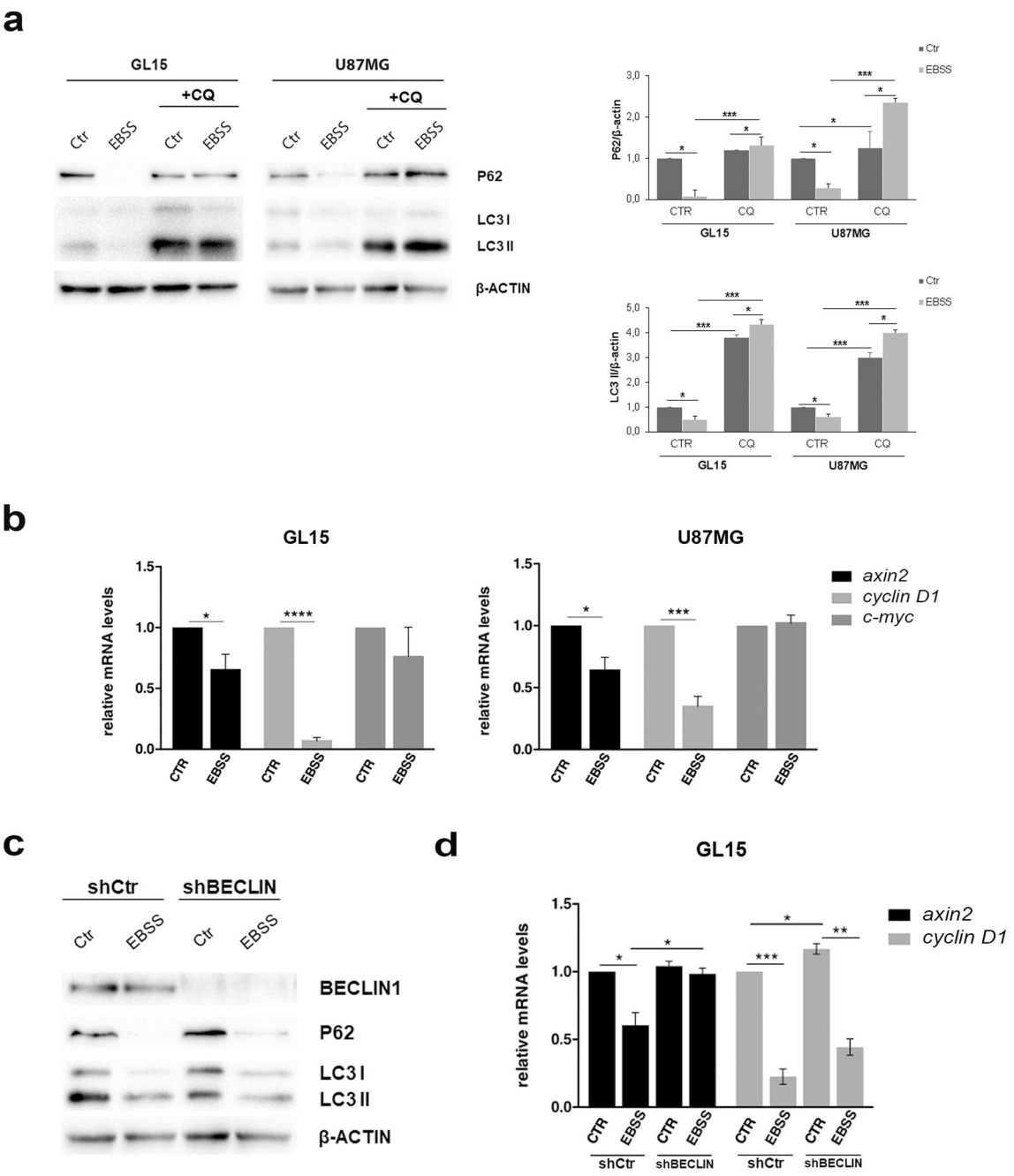

Fig. 1. Autophagy induction reduces Wnt signalling. (a) GL15 and U87MG cells were cultured in DMEM (Ctr) and aminoacid- and serum- free medium (EBSS) for 18 h, in presence or not of 20 μM Chloroquine (CQ). Western Blot analysis of autophagy markers P62 and LC3 I/II was used to check autophagy induction. β-ACTIN was used as loading control. The blots are representative of three independent experiments. (b) mRNA expression levels of *axin2*, *cyclin D1* and *c-myc* obtained by qPCR on GL15 (left panel) and U87MG (right panel) cultured in DMEM (Ctr) and aminoacid- and serum- free medium (EBSS) for 18 h. The graphs represent the mean ± SE of three different experiments. Statistical significance: * $P \leq .05$; *** $P \leq .001$; **** $P \leq .0001$. (c) GL15 were transduced with a scramble RNA (shCtr) or with a BECN 1-directed shRNA (shBECLIN) and were grown in DMEM (Ctr) or EBSS for 18 h. Western Blot analysis of autophagy markers P62 and LC3 I/II was used to check autophagy induction. An antibody specific for BECLIN1 was used in order to check the silencing efficiency. β-ACTIN was used as loading control. The blots are representative of three independent experiments. (d) mRNA expression levels of *axin2* and *cyclin D1* obtained by qPCR on GL15 cells after lentiviral infection with scramble RNA (shCtr) or BECN-1-directed RNA (shBECLIN) grown in DMEM (Ctr) or EBSS for 18 h. The graph represents the mean ± SE of three different experiments. Statistical significance: * P ≤ .05; ** $P \leq .01$; *** $P \leq .001$.

3. Results

3.1. Wnt/β-catenin signalling is negatively regulated by autophagy in GBM cells

In order to analyse the effect of autophagy induction on Wnt signalling in GBM cells, autophagy was induced by means of 18 h aminoacid- and serum-starvation (EBSS treatment) in GL15 and U87MG cells, as previously described [8] and as shown in Fig. 1a. The inhibitor of autophagosome-lysosome fusion Chloroquine (CQ) was employed to confirm an ongoing autophagy flux [26], thus demonstrating that, in absence of CQ, LC3 is fully converted to LC3 II form, that, in turn, is degraded into lysosomes, as well as p62, in glioma cells cultured in EBSS for 18 h (Fig. 1a).

qPCR analysis has been performed to measure the relative mRNA levels of the β-catenin target genes *axin2*, *cyclinD1* and *c-myc* in both control and in EBSS-starved cells. We observed a significative reduction in *axin2* and *cyclinD1* mRNA levels in both EBSS-treated GL15 and U87MG cell lines, in respect to control cells, grown in nutrient-rich medium (Fig. 1b). Similar results were obtained when autophagy was induced by culturing GBM cells in the presence of the mTOR inhibitor Torin1 (Torin) (Supplementary Fig. S1a and b). Differently from other cellular systems, we could not detect any significative variation of *c-myc* mRNA levels in cells grown in EBSS or in Torin presence (Fig. 1b and not shown). In order to prove the role of the autophagic process in promoting β-catenin de-activation upon nutrient starvation, we knocked down the autophagy regulator Beclin1, by means of specific siRNA. GL15 cells lacking of Beclin1 showed a reduced basal autophagy levels, and a decreased capability to accumulate LC3II and to degrade p62 substrate upon nutrient deprivation (Fig. 1c). In addition, the reduction of β-catenin target genes mRNA, that we observed in control cells upon nutrient deprivation, was prevented by Beclin1 silencing, almost completely for *axin2* and, at lesser extent for *cyclinD*, that is much more responsive to starvation (Fig. 1d). Moreover, a slight, but significative, increase of the basal level of *cyclinD* mRNA was observed in Beclin1-defective cells in nutrient-rich conditions thus further suggesting role for autophagy in modulating Wnt/β-catenin signalling (Fig. 1d).

3.2. Dishevelled protein is degraded upon autophagy induction

Dishevelled (Dvl) protein is a component of the protein family that mediates Wnt pathway by binding to Frizzled receptor and by inhibiting the APC-containing destruction complex [27]. Negative modulation of Wnt signalling has been linked to Dvl degradation upon autophagy induction in HEK293 and in colorectal cancer cells [23].

We analyzed the expression level of Dvl2 and, similarly to what observed in HEK293 cell line, both GL15 and U87MG cells showed an appreciable reduction of the protein upon autophagy induction (Fig. 2a and Supplementary Fig. S1c). The same result was obtained in GBM130 primary cells grown in EBSS medium (Fig. 2a).

On the other hand, no variation in Dvl2 protein levels was observed in Beclin1-devoid cells, thus demonstrating that autophagy induction is necessary for Dvl2 degradation (Fig. 2b).

3.3. The expression level of the transcriptional active β-catenin decreases upon autophagy induction

In 2013, Petherick et al. have demonstrated that nutrient deprivation leads to proteasome-independent and autophagosome-mediated β-catenin degradation in colorectal carcinoma cells, that was likely responsible for Wnt/β-catenin signalling inactivation [22]. Moreover, Cheng et al. have been shown that Niclosamide induces autophagy and β-catenin degradation in GBM cells [24] . To test if β-catenin was degraded upon nutrient deprivation in glioma models, we analyzed β-catenin amount by western blot, and we did not observe any difference in the total protein levels between control and 18 h-starved GL15 and U87MG cell lines. However, by means of an antibody specifically directed against the de-phosphorylated (S37/T41) β-catenin, we observed a significative decrease of the nuclear transcriptionally active protein pool in autophagic glioma cells in respect to control cells (Fig. 3a and Supplementary Fig. S1c). This reduction of the active β-catenin pool was not observed in Beclin1-devoid cells, thus suggesting a direct involvement of Beclin1-dependent autophagy in β-catenin transcriptional activity (Fig. 3b).

3.4. Autophagy induction induces β-catenin relocalisation in GBM cells

In line with the so far obtained results, we observed, by means of subcellular fractionation experiments, a significative reduction of the β-catenin nuclear fraction and a slight increase in the cytosolic one, upon 18 h of EBSS- and Torin- culturing in the cell lines analyzed (Fig. 4a and Supplementary Fig. S1d). Interestingly, this starvation-dependent reduction in the β-catenin nuclear fraction upon starvation was completely prevented in GL15 cells lacking of Beclin1 protein although no difference in the β-catenin nuclear basal level was observed (Fig. 4b). In order to verify a possible re-localisation of the cytosolic β-catenin pool, we analyzed the protein's expression pattern in GL15 and in U87MG glioblastoma cells by immunocytochemistry. Widespread expression of the protein within the cells was observed in control conditions (Fig. 4c, upper panels), whereas a relevant accumulation of β-catenin in subplasma membrane areas was observed in cells cultured for 18 h in EBSS (Fig. 4c, bottom left and middle panels) or in Torin presence (Supplementary Fig. S1e, middle panels and not shown), but not in Beclin1-silenced GL15 cells (Fig. 4c, bottom right panel). Strikingly, we observed a clear enlargement of the cells and the appearance of cell-cell

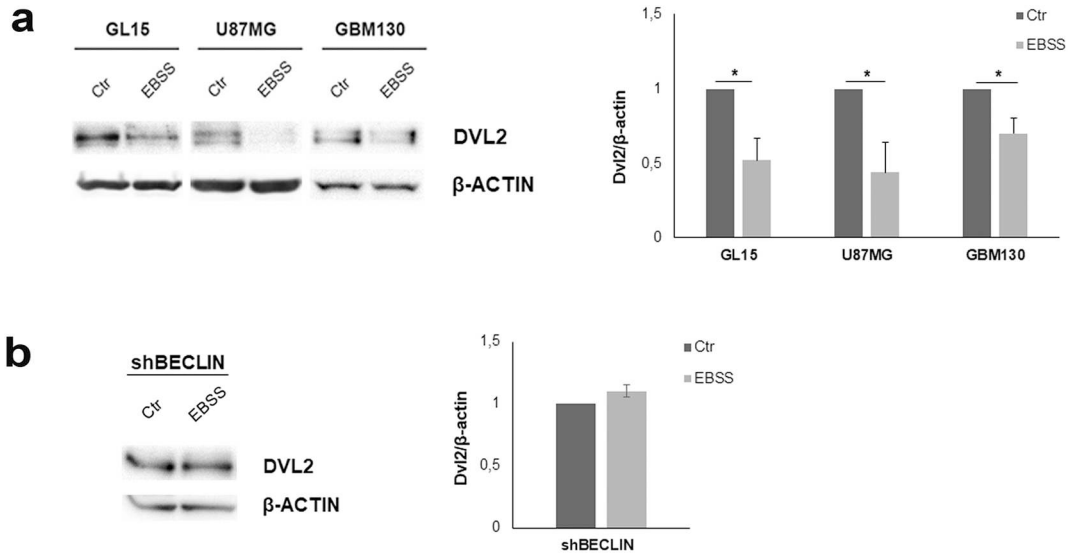

Fig. 2. Autophagy regulates Dishevelled protein levels in GBM cell lines. Western blotting of protein extracts from GL15, U87MG and GBM130 (a) and shBECLIN1 GL15 cells (b), cultured in DMEM (Ctr) or EBSS, was performed by using a specific antibody for Dishevelled 2 (DVL2). β-ACTIN was used as loading control. The graphs represent the mean ± SE of three different experiments. Statistical significance: * $P < .05$ Student *t*-test.

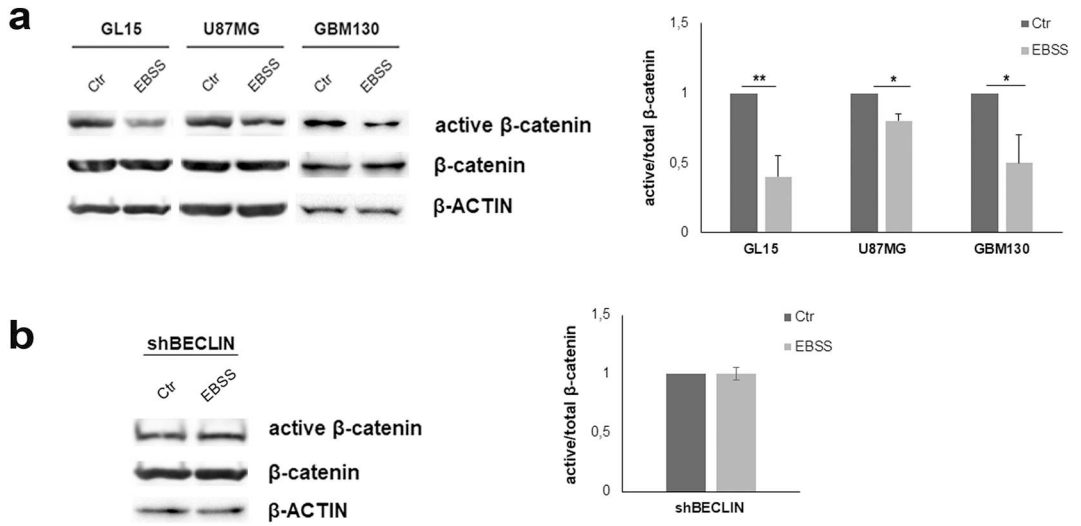

Fig. 3. β-catenin activity is reduced in GBM cells upon autophagy induction. Western blotting of protein extracts from GL15, U87MG, GBM130 (a) and shBECLIN1 GL15 cells (b), cultured in DMEM (Ctr) or EBSS was performed by using specific antibodies for the total (β-catenin) and the dephosphorylated (S37/T41) form of β-catenin (active β-catenin). β-ACTIN was used as loading control. The graphs indicate the relative ratio between active/total β-catenin and represent the mean ± SE of three different experiments. Statistical significance: * P < .05; ** P ≤ .01 Student *t*-test.

contacts, highlighted by β-catenin signal, that were almost completely absent in control cells (Fig. 4c and Supplementary Fig. S1e).

3.5. β-catenin associates with N-cadherin upon autophagy induction

Based on the evidence of β-catenin relocalisation in sub-plasma membrane areas, that suggested a possible interaction with membrane proteins involved in cell-cell interactions, we then analyzed N-cadherin localisation within glioma cells in our experimental conditions. Confocal analysis showed a diffuse N-cadherin intracellular localization in U87MG and GL15 control cells (Fig. 5, left upper panels). Conversely, in EBSS-cultured cells, N-cadherin staining overlapped with β-catenin signal in correspondence of cell-cell contacts (Fig. 5). The same result was observed in Torin-treated U87MG (Supplementary Fig. S1e, bottom panels). This result suggested that autophagy induction promotes the association between β-catenin and N-cadherin in glioma cells, and that this event is likely responsible for newly formed N-cadherin-mediated cell-cell junctions.

4. Discussion

A number of studies report Wnt signalling as aberrantly activated in GBM, thus promoting tumour growth and propagation, by transcriptional activation of multiple target genes, involved in both proliferation and invasion [13]. In the recent years, it has been reported that in HEK293 and in colorectal cancer cells, Wnt signalling could be attenuated under nutrient deprivation by accelerating autophagy-mediated Dvl degradation [23]. In addition, in nutrient-stressed colorectal cancer cells, β-catenin can be per se a direct target of autophagic degradation, in a proteasome-independent manner [22]. β-catenin down-regulation and Wnt pathway inhibition has been also observed in a GBM cellular model treated with the anti-helminthic drug Niclosamide, that is also able to induce autophagy, although the mechanism of action remains to be elucidated [24].

Here we demonstrate that autophagy induction negatively regulates Wnt/β-catenin signalling in GBM cellular models. A robust autophagy induction was obtained by nutrient-depriving GBM cells or by culturing them in presence of the mTOR inhibitor Torin1 for 18 h, as demonstrated by LC3 conversion and p62 degradation. Similarly to other cellular models, no variation in Beclin1 protein level was observed in GBM cells upon autophagy induction, but, conversely, genetic silencing of Beclin1 largely prevented autophagy execution thus demonstrating a Beclin 1-dependent autophagy occurrence.

We report, for the first time, that nutrient starvation and m-TOR inhibition strongly attenuate *cyclinD1* and *axin2* transcription, through an autophagy-dependent mechanism, as suggested by the rescue observed in Beclin1 silenced cells. Conversely, *c-myc* transcription (and *n-myc* too, data not shown) is not affected by nutrient starvation or by m-TOR pharmacological inhibition, thus suggesting a Wnt-independent regulation of *myc* genes in GBM cells. Of note, we did not see any rescue effects of nutrient deprivation on Wnt/β-catenin signalling, when the PI3K inhibitors Wortmannin and 3-methyladenine (3-MA) were employed to chemically block autophagy induction, although β-catenin appeared mainly localized in the nucleus in its active form (not shown). We hypothesize that Wortmannin and 3-MA are not suitable for testing the effects of autophagy inhibition in GBM cells, as they can have, per se, effects on several intracellular processes and can even promote autophagy in some systems [26].

The decrease in Dvl and in axin2 expression that we observed could explain, at least partially, the impairment of Wnt/β-catenin signalling obtained.

We also found that autophagy activation by nutrient deprivation or by m-TOR inhibition does not result in β-catenin degradation in GBM cells, as observed in a colorectal model, but, surprisingly, we observed that autophagy promotes β-catenin relocalisation within the cell. In fact, we observed a decrease of the β-catenin nuclear fraction, coupled to an accumulation of the cytosolic one. In particular, immunofluorescence experiments demonstrated a different signal distribution of the protein among nutrient rich- and nutrient poor- conditions. Upon autophagy induction, β-catenin appears mainly localized in sub-membrane areas where it decorates the cell-cell contacts which are much more evident than in control cells. The appearance of an epithelial-like phenotype of these cells was confirmed by the co-localisation of β-catenin with N-cadherin, in correspondence of the cell-cell contacts.

By our previous observations, we know that N- and R-cadherin mRNA level increase in glioma cells upon autophagy induction, likely as consequence of Snail1/2 down-regulation [8]. The role of N-cadherin in EMT is not completely understood. In epithelial tumours (carcinomas), in fact, the cadherin switch, from *E*- to N-isoform, is thought as a hallmark of EMT occurrence [28,29], but the contribution of cadherin

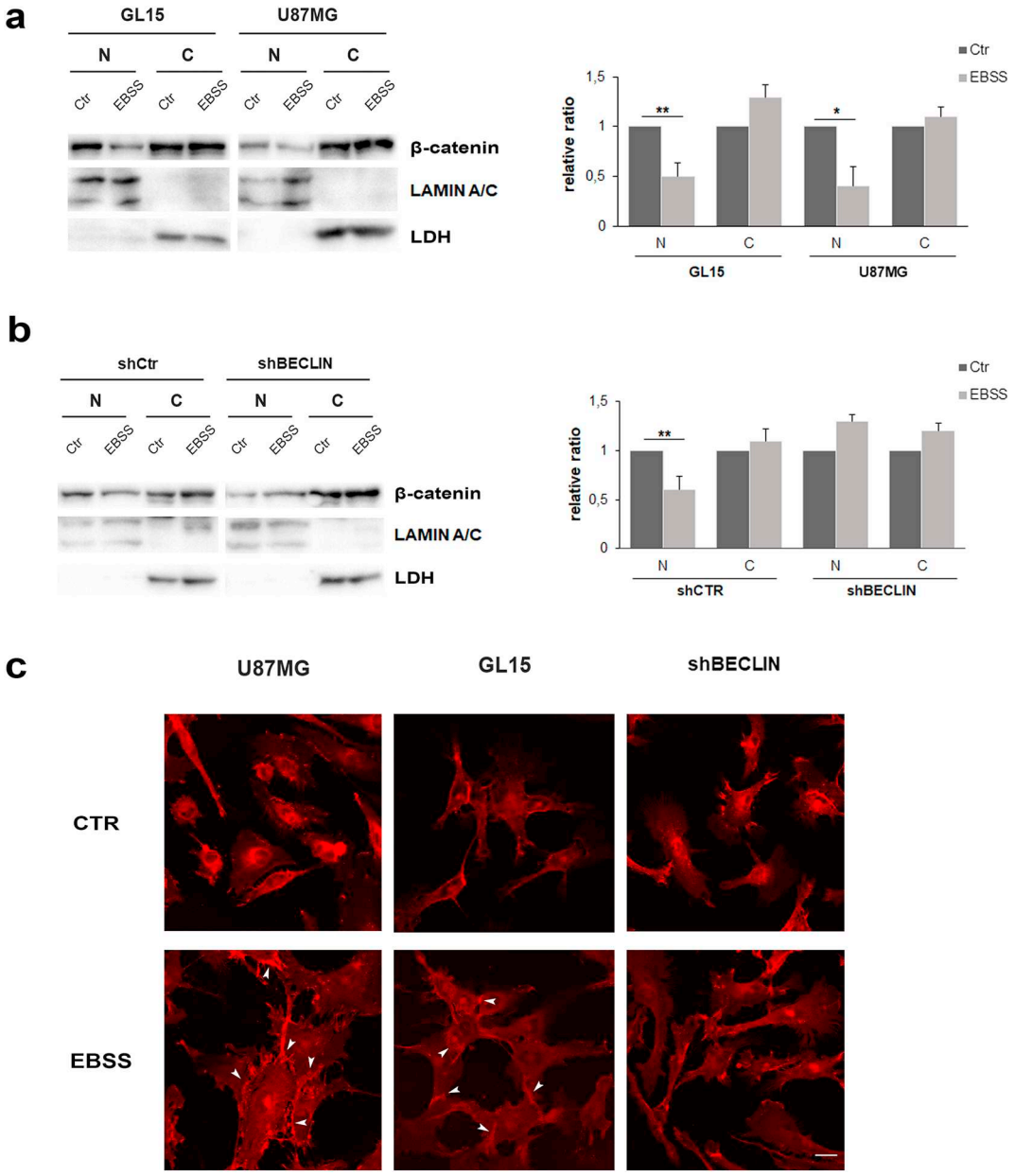

Fig. 4. Autophagy induces β-catenin subcellular relocalisation. (a) After subcellular fractionation experiments in GL15 and U87MG cultured in DMEM (Ctr) or EBSS for 18 h, Western blotting of protein extracts was performed by using a specific antibody for β-catenin. Lamin A/C and LDH were used as loading controls for nuclear and cytosolic fractions, respectively. The graph indicates the ratio between β-catenin and Lamin A/C (for nuclear expression) and β-catenin and LDH (for cytosolic expression) in EBSS compared to control cells in three independent experiments. N, nuclei; C, cytosol. Statistical significance: * $P < .05$; ** $P \leq .01$ Student *t*-test. (b) The same experiment was performed in GL15 transduced with a scramble RNA (shCtr) or with a BECN 1-directed shRNA (shBECLIN) grown in DMEM (Ctr) or EBSS for 18 h. The graph indicates the ratio between β-catenin and Lamin A/C (for nuclear expression) and β-catenin and LDH (for cytosolic expression) in EBSS compared to control cells in three independent experiments. N, nuclei; C, cytosol. Statistical significance: ** $P \leq .01$ Student *t*-test. (c) U87MG, GL15 and shBECLIN1 GL15 cells grown in DMEM (Ctr) or EBSS medium were subjected to immunocytochemistry and confocal analysis for β-catenin localisation (red). Arrowheads indicate cell-cell contacts. Scale bar, 30 μM. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

isoforms in glioma EMT is less documented. It has been demonstrated that expression of N-cadherin inversely correlates with the invasive behaviour of GBM cells [30,31], and the instability and disorganisation of cadherin-mediated junctions are likely associated to GBM invasive behaviour [32,33]. Our data are consistent with a newly acquired role for β-catenin in supporting the EMT reverse process (mesenchymal-to-epithelial-like transition, MET) observed in glioma cells when they are forced to undergo autophagy [8].

The role of autophagy in GBM pathogenesis is still a matter of debate [34,35]; autophagy induction has been observed in GBM in response to radio- and temozolomide-based therapy and even though a number of clinical trials aimed at inhibiting autophagy execution, mainly by Chloroquine, have been launched, others directed to inhibiting mTOR pathway and thus activating autophagy, are ongoing [36,37]. Intriguingly, we observed that, in addition to impair cell migration and invasion [8], autophagy induction also results in a significative arrest of cell proliferation, as measured by FACS analysis, cell counting and protein quantification (not shown). Although further studies are necessary to better dissect autophagy role in GBM progression, taken together our findings support the idea that autophagy modulation could represent a potential therapeutical strategy to contrast GBM invasiveness.

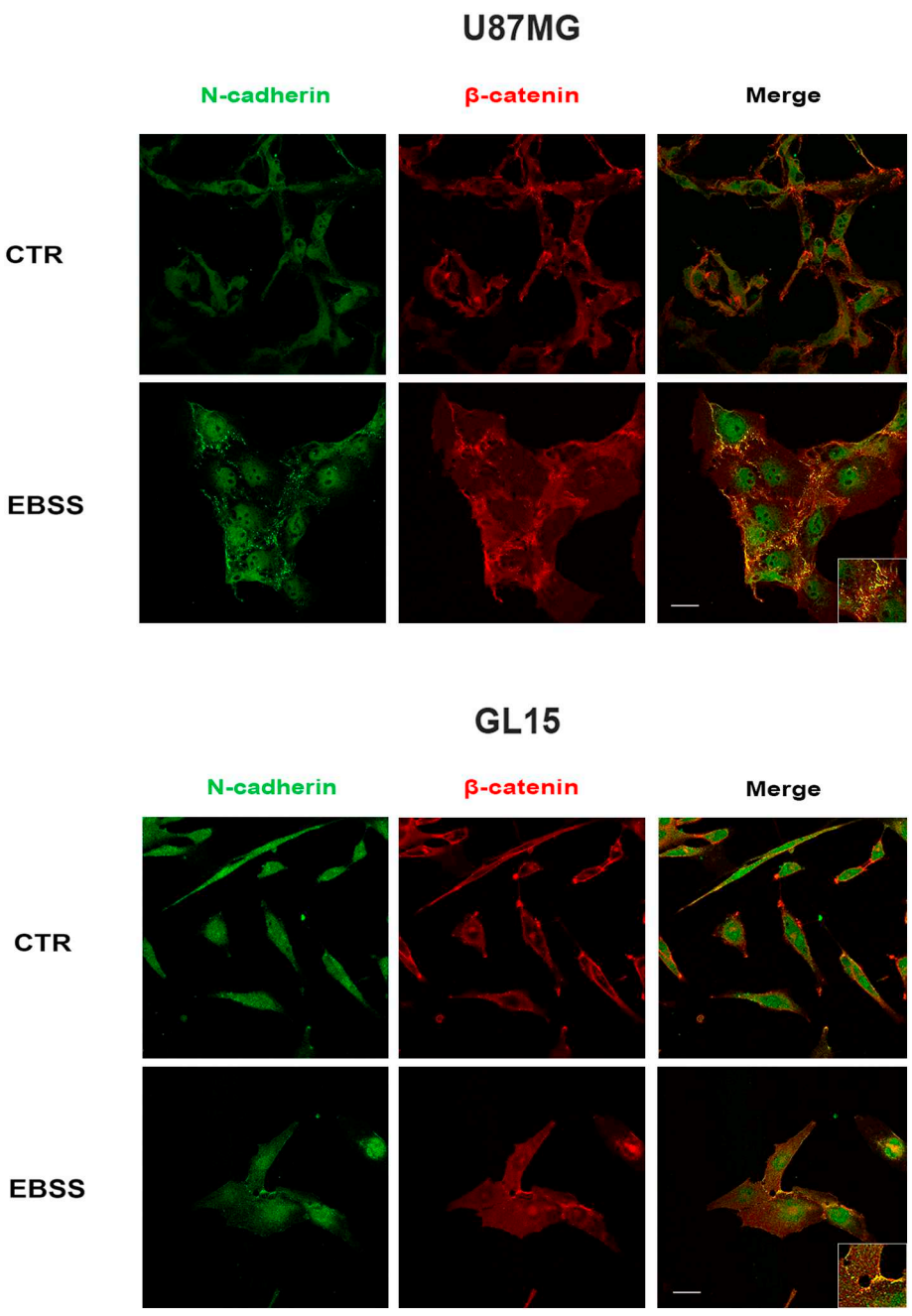

Fig. 5. β-catenin and N-cadherin colocalise upon autophagy induction. U87MG and GL15 cells grown in DMEM (Ctr) or EBSS medium were subjected to immunocytochemistry and confocal analysis for N-cadherin (green) and β-catenin (red) localisation. Inset containing higher magnification views of the merge images are also shown. Scale bar, 30 μM. Colocalisation index was assessed by calculating the Pearson's correlation coefficient *r* of at least 10 cells analyzed in two different experiments (mean *r*: Ctr, 0.20 ± 0.02; EBSS, 0.72 ± 0.07 for U87 and mean *r*: Ctr, 0.15 ± 0.02; EBSS, 0.66 ± 0.05 for GL15). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

5. Conclusions

GBM is the most common and lethal adult brain tumour and it is characterized by a highly invasive behaviour. An aberrantly activated Wnt/β-catenin signalling is thought responsible for GBM invasion capability, beside for tumour proliferation, and stemness maintenance.

We have found that autophagy negatively regulates Wnt/β-catenin signalling in glioblastoma cellular models. Moreover, we observed that β-catenin relocalises within the GBM cell and associates with N-cadherin, thus contributing to mesenchymal-to-epithelial-like transition. The findings reported here add important clues for the definition of possible therapeutical strategies, targeting the autophagic process, to contrast GBM progression.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cellsig.2018.10.017.

Author contributions

S.D.B. conceived the project. B.C. and S.D.B. designed experiments. M.C.[2] designed and performed Real Time experiments. B.C. and F.F. carried out biochemical and immunofluorescence experiments and prepared figs. G.D. and M.C.[3,4] obtained primary cells and contributed to design and analyse experiments. A.S. provided tumour biopsies for primary cultures. B.C. and S.D.B. wrote the paper with input from all authors. C.L. and F.C. supported the research and critically read the paper.

Conflict of interest declaration

The authors declare no conflict of interest.

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