Defining the Functional Role of the Autophagy Regulatory Gene Ambra-1 in Melanoma invasion and Metastasis

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Introduction and Aims

Cutaneous malignant melanoma, the most aggressive form of skin cancer, arises from the malignant transformation of epidermal melanocytes[1]. Although curable at early stages via surgical incision there is however, no effective treatment for malignant disease.

Autophagy is an essential cellular mechanism whereby the degradation of surplus proteins and damaged organelles sustains cellular metabolic activity and cell survival[2, 3]. Paradoxically in cancer, autophagy acts both as a tumour suppressor mechanism while also promoting tumour progression. Furthermore, autophagy appears to contribute to chemoresistance through counter-acting apoptotic signaling[4]. Current therapeutic strategies have therefore focused on the inhibition of autophagy to promote cell death.

Ambra-1 is a major autophagy regulatory protein. Since Ambra-1 contributes to enhanced autophagy and may fuel tumour invasion and migration, modulation of Ambra-1 may therefore represent a novel therapeutic strategy.

The aim of the current study was thus to: ***define the functional role of Ambra-1 in melanoma invasion and metastasis.***

To this aim, the principle objectives were to:

- Investigate the effect of Ambra-1 modulation in metastatic melanoma cell lines on cell **migration** in a 2D cell culture scratch assay.
- Investigate the effect of Ambra-1 modulation on the ability of melanoma cells to form spheroids in 3D and invade into collagen gels as a measure of **invasion**.

Materials and Methods

METASTATIC MELANOMA CELL LINES

Human A375 metastatic melanoma cells, or A375 cells retrovirally transduced to stably over-express Ambra-1 or in which Ambra-1 had been stably knocked down via lenti viral short-hairpin RNAi mediated interference were grown and maintained in DMEM supplemented with 10% FCS, 5% P/S (culture medium)

SCRATCH ASSAY

Cells were seeded at a density of $1.5X10^5$ cells/well in in 24 well flat bottom tissue culture plates and left overnight to adhere. A pipette tip was used to 'scratch' a straight line down the middle of the well in order to create a gap between cells. Cells were imaged hourly using a Nikon Biostation CT for 48 hours and the area of the void measured using ImageJ software and the percentage gap closure determined every 3 hours.

COLLAGEN INVASION ASSAY

1.5% low melting point (LMP) or bacterial agar were dissolved by heating in 1XPBS 100µl of agar solution was applied to each well of a 96 flat well plate before cells were seeded on top at a density of $5X10^3$/well in 100µl of culture medium for 96 hours to allow spheroid formation. 200µl Collagen was then added to 24 well plates and after 5 minutes the best 3 spheroids of each cell type removed and re-suspended in 300µl collagen mix before addition to the collagen coated wells. After 15 minutes incubation, 1 ml of culture media was added to each well and images of spheroids acquired every 24 hours for 5 days. The area of the spheroid was measured using Image J.

Results 1: Modulation of Ambra-1 impacts on cell migration

Stable over-expression of Ambra-1 had little effect on the rate of void closure compared to control cells over-expressing control β galactosidase (Figure 1) over 48 hours. However, compared to cells stably expressing control siRNA, stable knockdown of Ambra-1 resulted in a slower rate of void closure over 24 hours but which at 48 hours was comparable to the control. Collectively these data suggest down-regulation of Ambra-1 may impair melanoma cell migration.

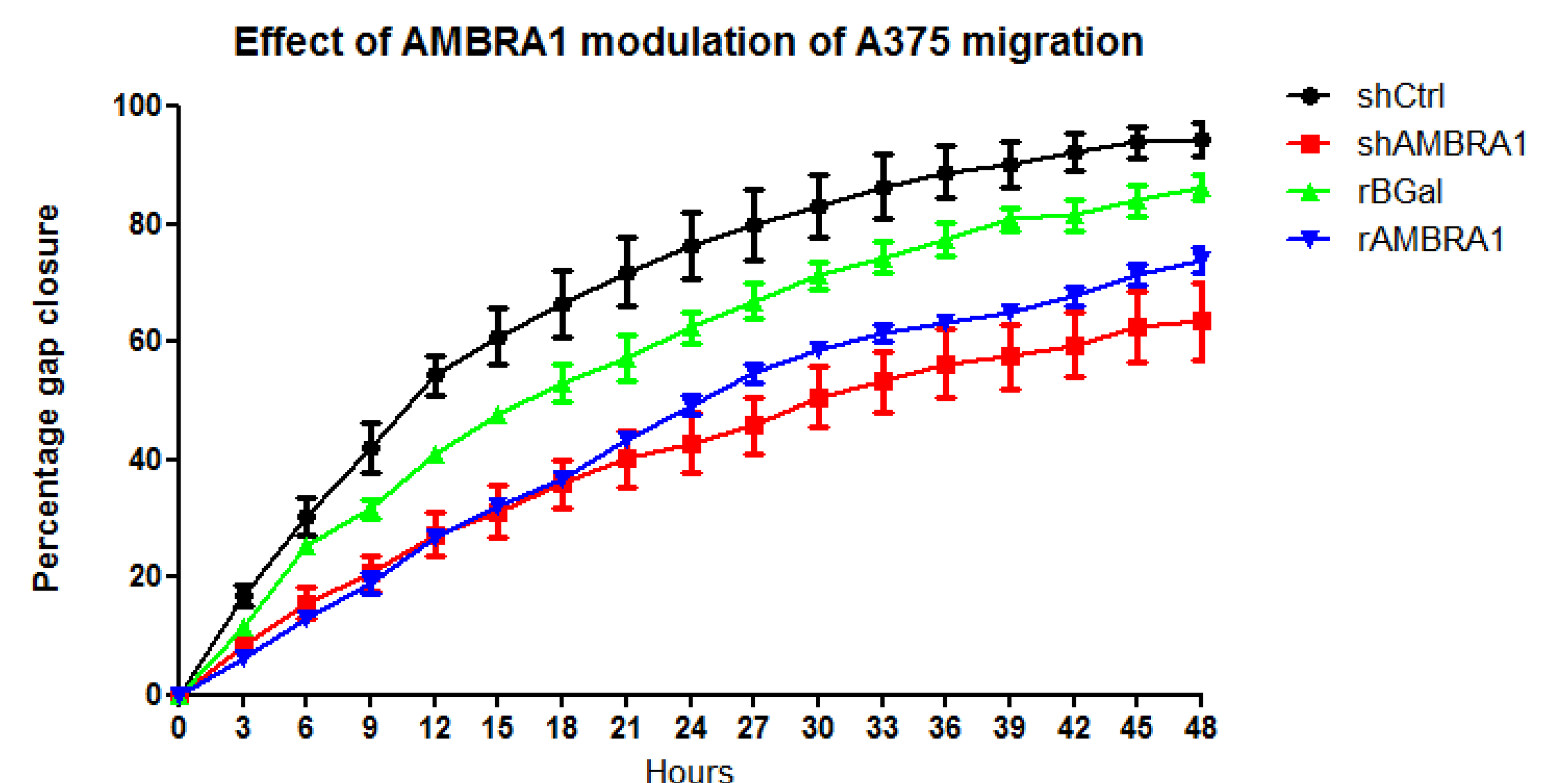

Figure 1: Percentage gap (void) closure over 48 hours of A375 cells expressing control shRNA (shCtrl, black line), or shAmbra-1 (ShAMBRA1, red line), or A375 cells over-expressing β-galactosidase control (rBGal, green line) or Ambra-1 (rAMBRA1, blue line) over 48 hours of culture. Each point is the mean of 3 voids ± SD.

Results 2: Modulation of Ambra-1 impacts on cell invasion

Time course studies of spheroid invasion by A375 cells in which Ambra-1 had been stably knocked down demonstrated increased invasion (by spheroids derived from 1.5% LMP agar) into collagen over 4 days (Figure 2 a & B) compared to spheroid invasion seen by control or A375 cells over expressing Ambra-1 (Figure 2B). In addition although 1.5% LMP agar resulted in optimal spheroid formation, spheroids derived from A375 cells with stable knockdown of Ambra-1 in 1.5% bacterial agar also resulted in increased invasion (Figure 2B red square). Over-expression of Ambra-1 also appeared to increase spheroid invasion, although at a relatively lower rate compared to the effect derived with Ambra-1 knockdown (Figure 2B). Collectively these data suggest knockdown of Ambra-1 increases tumour invasion.

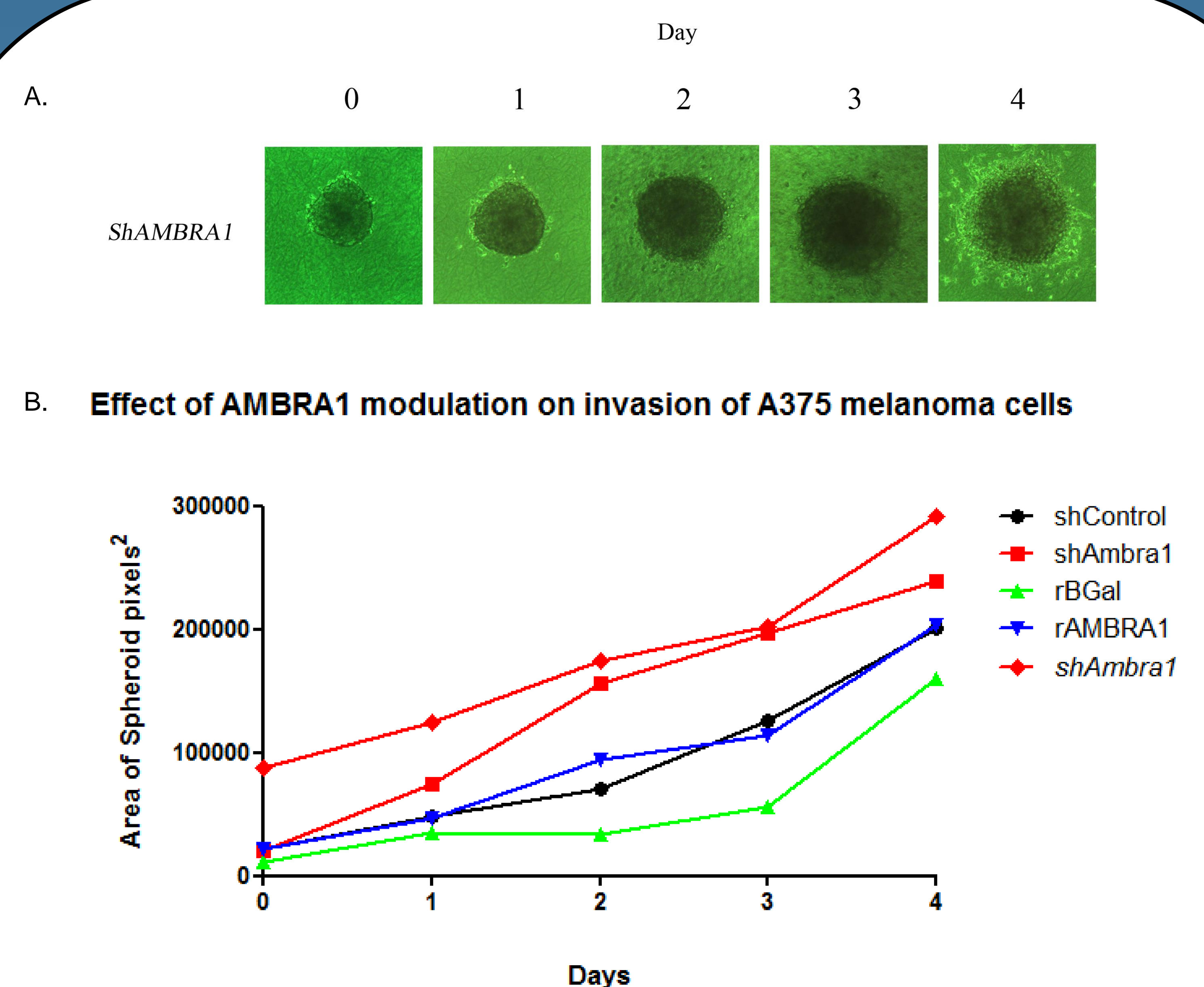

Figure 2: Pictomicrographs of spheroid growth progression. Spheroid growth of the A375 cells expressing shAmbra-1 (ShAMBRA1) over the 4 days. This spheroid was grown in LMP agar (A). Area of spheroids of the A375 cells expressing control shRNA (shCtrl), or shAmbra-1 (ShAMBRA1, *ShAMBRA1*), or A375 cells over expressing β- galactosidase control (rBGal) or Ambra-1 (rAMBRA1) over 4 days(B).

Conclusions

- Knockdown of Ambra-1 decreases metastatic melanoma cell migration.
- Knockdown of Ambra-1 increases metastatic melanoma spheroid invasion

Summary

Loss of Ambra-1 decreases cell migration and increases invasiveness of metastatic melanoma cells suggesting modulation of Ambra-1 may be a novel therapeutic strategy.

References

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