Hilary S. Dorward, Alice Du, Maressa A. Bruhn, Joseph Wrin, Jinxin V. Pei, Andreas Evdokiou, Timothy J. Price, Andrea J. Yool, and Jennifer E. Hardingham
Pharmacological blockade of aquaporin-1 water channel by AqB013 restricts migration and invasiveness of colon cancer cells and prevents endothelial tube formation in vitro.
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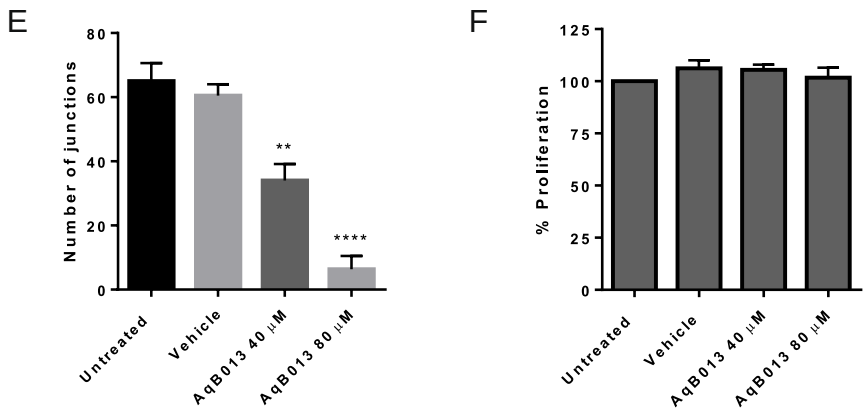

Fig. 3 Angiogenesis assay. HUVEC tube-forming assay measured by the number of junctions: **a**, untreated HUVEC; **b**, vehicle treated HUVEC; **c**, HUVEC treated with 40 μM AqB013; **d**, HUVEC treated with 80 μM AqB013 (40 x magnification, scale bar = 0.5 mm); **e**, graph shows significant inhibition of endothelial tube formation by AqB013 at 40 μM and 80 μM, **$p < 0.01$, ****$p < 0.0001$ respectively (ANOVA). **f**, AqB013 treatment had no effect on proliferation of HUVECs

models and may thus serve as a potential target for small molecule inhibitors to treat cancer in subgroups expressing AQP1. The *in vitro* testing of drugs is the first step in establishing the efficacy of targeting specific molecules in abrogating the migration and invasion of cancer cells, or in suppressing angiogenesis.

In this study, inhibition of AQP1 by the inhibitor AqB013 was effective in reducing migration (wound closure assay) and invasion (spheroid formation assay) in the high AQP1-expressing HT29 cells, while not affecting migration or invasion in HCT-116 cells that had much lower expression of AQP1. As both untreated and vehicle-treated HT29 and HCT-116 cells showed similar efficiency of wound closure and invasion, these results suggest that AQP1 was indeed the target of inhibition. In breast cancer cells, AQP5 polarizes to the leading edge of migrating MDA-MB-231 cells, and that knockdown of AQP5 in these cells significantly suppressed cell migration velocity in narrow channels [23]. Similarly, knockdown of AQP5 in MCF7 breast cancer cells resulted in significantly reduced proliferation and migration [24]. However we have shown that the expression of AQP5 in HCT-116 is low (Additional file 1), similar to that of AQP1, suggesting that in these cells an

alternative mechanism of migration is used which would explain why these cells are resistant to the inhibitory effect of AqB013. Migration in HCT-116 cells that express low amounts of AQP1 and AQP5 may be enhanced by expression of the calcium activated chloride channel TMEM16A as it has recently been reported that the high metastatic-potential colon cancer cell lines HCT-116 and SW620 express TMEM16A while primary colon cancer cell lines HCT8 and SW480 cells do not. Knockdown of TMEM16A by short hairpin RNA in SW620 resulted in significantly reduced migration in wound-closure assays [25]. In addition HCT-116 cells have been shown to have high levels of micro RNA 224 which has recently been shown to activate the Wnt-β catenin pathway to promote migration and invasion of HCT-116 cells [26, 27], rendering the cells resistant to the effects of AQP inhibition.

AQP1 has been shown to have dual water channel and gated ion channel functions [16, 28, 29]. The AQP1-mediated cationic conductance has been implicated in influencing rates of net fluid transport in primary cultures of choroid plexus [30], and similarly this mechanism may be responsible for regulating net fluid flux in migrating epithelial and endothelial cells. However work by the Yool group has previously shown that endogenous chloride conductance in *X. laevis* oocytes is not blocked by AqB013. Furthermore in mouse intact gastric antral muscle, the addition of AqB013 did not change the resting membrane potential and had no substantial effect on the rhythmic electrical conduction properties [18]. Taken together these data suggest that the effect of AqB013 on impeding the migration of human colon cancer cells and endothelial cells expressing AQP1 is mediated by blocking the water channel activity of AQP1.

Conclusions

These studies have shown clear links between AQP1 activity and cancer cell migration and invasion, and endothelial cell tube–forming capacity, indicating the importance of characterising suitable AQP1 blockers. This study provides preliminary data showing that the AQP1 inhibitor AqB013 abrogates endothelial tube formation and reduces cancer cell migration and invasion and will be further investigated in an *in vivo* mouse xenotransplant model of human colon cancer. Small molecule pharmaceuticals have an established therapeutic use and as this new drug is a modification of bumetanide, it should be well-tolerated in cancer patients with far fewer side-effects than from currently used chemotherapeutic drugs. Furthermore, in view of the documented role of AQP1 in murine tumour angiogenesis [10, 12], it is envisaged that in metastatic CRC patients, AQP1 inhibitors may have a role combined with anti-vascular endothelial growth factor (VEGF) therapy, or as an alternative anti-angiogenesis therapy in cases that become resistant to anti-VEGF therapy. The inhibition of AQP1 clinically may slow down the progression of CRC, increasing the window for optimal treatment resulting in better survival outcomes, particularly in early stage cases where micro-metastatic disease is present.

Additional file

Additional file 1: Expression of AQP1 and AQP5. qPCR ($2^{-\Delta Ct}$) results normalised to reference gene PMM1. (PDF 95 kb)

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

JH, AY, AE, TP made substantial contributions to conception and design of the study and to interpretation of data; HD, JH made substantial contributions to acquisition and analysis of data and co-wrote the manuscript. HD, AD carried out the angiogenesis assays; HD, MB and JP carried out the migration assays; HD carried out the invasion and proliferation assays, JW performed the immunohistochemistry. All authors reviewed and approved the manuscript.

Author details

[1]Molecular Oncology, Basil Hetzel Institute, The Queen Elizabeth Hospital, Woodville, SA, Australia. [2]Discipline of Physiology, School of Medicine, University of Adelaide, Adelaide, SA, Australia. [3]Disciplines of Surgery and Orthopedics, School of Medicine, University of Adelaide, Adelaide, SA, Australia. [4]Medical Oncology, The Queen Elizabeth Hospital, Woodville, SA, Australia. [5]School of Medicine, University of Adelaide, Adelaide, SA, Australia. [6]Level 1, Basil Hetzel Institute, The Queen Elizabeth Hospital, 28 Woodville Road, Woodville, SA 5011, Australia.

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