Supplementary Note for the Report on
the Computational Modelling of Aquaporin Co-regulation in Cancer

Discretisation Tables

1 Chae, Y. K. *et al.* Expression of aquaporin 5 (AQP5) promotes tumor invasion in human non small cell lung cancer. *PLoS One* **3**, e2162, doi:10.1371/journal.pone.0002162 (2008).

- Human NSCLC samples: 35.3% expressed AQP5 (immunostaining score>0; no AQP5 in infiltrating lymphocytes)
- Forced overexpression of AQP5 promotes BEAS-2B (human lung epithelial) and NIH3T3 (murine fibroblast) *in vitro* invasion (assay) (***NB*** not cancer cell line)
- Forced overexpression of two mutants, one blocking phosphorylation on Ser156 (**S156A**), the other blocking membrane trafficking (**N185D**), do not
- Active binding of BEAS-2B^{+AQP5} extracts to active c-Src SH3 domain
- FISH do not show AQP5 gene amplification
- ***NB*** **check proliferation** is low (at least =1) at initial stages (p53-null OK)

AQP5	0	2
c-Src	0	2
Invasion	0	2

2 Kang, S. K. *et al.* Role of human aquaporin 5 in colorectal carcinogenesis. *Am J Pathol* **173**, 518-525, doi:10.2353/ajpath.2008.071198 (2008).

- Human CR cancer samples: 62.8% expressed AQP5 (immunostaining; 50% score>2)
- siRNA-AQP5 decreases HCT116 (colon cancer) proliferation and reduces p-ERK
- HCT116 with transfected AQP5 WT or N185D sees increase in proliferation (60%/30%), but not S156A (***NB*** only control with empty vector, not no-vector)
- HCT116 with transfected AQP5 WT (only) sees **strong** increase in p-ERK blot
- echoed by another study in human NSCLC cell lines injected into mice
- HCT116 DOES contain wild-type p53

AQP5	0	1	2	2	
c-Src	0	1	2	1(F)	
ERK	1	2	3	2	
Proliferation	0	1	2	1	(p53 in)

3 Chae, Y. K. *et al.* Human AQP5 plays a role in the progression of chronic myelogenous leukemia (CML). *PLoS One* **3**, e2594, doi:10.1371/journal.pone.0002594 (2008).

- Human CML samples: 32% expressed AQP5 (null in normal bone marrow)
- K562 (CML) transfected with AQP5 shows higher proliferation & slightly higher p-Akt blot (***NB*** only control with empty vector, not no-vector)
- siRNA-AQP5 decreases proliferation, and, slightly, p-Akt blot (***NB*** only control with non-related siRNA, not no-siRNA)
- Higher caspase 9 activity and lower ATP concentration shown in LAMA-84 (CML) transfected with siRNA-AQP5 (***NB*** compared to control-siRNA)
- FISH shows no AQP5 gene amplification – could be secondary
- ***NB*** not considering the effect of BCR (BcrAbl) because of generic model

AQP5	0	1	2	
Akt	0	1	2	
Caspase-9	2	1	0	
Proliferation	0	1	2	(p53 in)
Apoptosis	**2**	1	0	

4 Warth, A. *et al.* Loss of aquaporin-4 expression and putative function in non-small cell lung cancer. *BMC Cancer* **11**, 161, doi:10.1186/1471-2407-11-161 (2011).

- Human NSCLC tumour samples
- Microarray & qRT-PCR: decrease in AQP1&4 expression in tumour compared to normal, while AQP5 level is **decreased (0.5-fold, qRT-PCR) or similar (microarray)**
- Adenocarcinoma predominantly expresses higher AQP1,3,4,5 than SCC
- Immunohistochemistry: well-differentiated samples show higher AQP4 expression
- Significant pro-tumorigenic effect of AQP4 could not be identified
- AQP4 has strong correlation with AQP1 (Pearson correlation 0.82)
- Long-term downregulation vs. overexpression of single AQP

AQP1	1**/2**	0**/1**	2**/1**	1	
AQP4	1	2	0	2	
Differentiation	1	2	0	2	
Invasion	1**/2**	0	2**/1**	1	
HIF1a	*1*	*2*	*0*	***1***	*(manipulate)*

(Probably because binding of HIF1a to AQP1 promoter complicates the case)
(Last column is the 'normal cell')

5 Mou, K. *et al.* AQP-4 in peritumoral oedematous tissue is correlated with the degree of glioma and with expression of VEGF and HIF-alpha. *J Neurooncol* **100**, 375-383, doi:10.1007/s11060-010-0205-x (2010).

- Human glioma tissue

- AQP4 expression elevated, and highly localised in peritumor
- AQP4 positively correlates with VEGF and HIF1a (Pearson correlation both 0.88)
- ***NB*** no investigation whatsoever on the mechanism (clinical article)

HIF1a (hypoxia)	0	1	2
AQP4	0	1	2

6 Yan, J. H. *et al.* p53-induced uncoupling expression of aquaporin-4 and inwardly rectifying K+ 4.1 channels in cytotoxic edema after subarachnoid hemorrhage. *CNS Neurosci Ther* **18**, 334-342, doi:10.1111/j.1755-5949.2012.00299.x (2012).

- Rat model with subarachnoid haemorrhage (SAH)
- High level of p53, AQP4 and p38 are seen with cytotoxic oedema after SAH
- Low level of Kir4.1 expression suggested uncoupling with AQP4
- Inhibition of p53 reversed the effect

p53	1/2	0(F)
AQP4	2	1

7 Jagirdar, R. *et al.* Gene expression profile of aquaporin 1 and associated interactors in malignant pleural mesothelioma. *Gene* **517**, 99-105, doi:10.1016/j.gene.2012.12.075 (2013).

- Data mining (*Oncomine*) of mesothelioma
- Positively correlated to TRIP6; negatively correlated to EFEMP2 (MBP1)
- CDKN2A (INK4A) deletion correlated to significant AQP1 downregulation
- INK4A can inhibit MBP1

INK4A	0	1	
AQP1	1	2	
Proliferation	2	1	(no hypoxia)
Fos/Jun	3	3	
MBP1	2	1	

8 Hoque, M. O. *et al.* Aquaporin 1 is overexpressed in lung cancer and stimulates NIH-3T3 cell proliferation and anchorage-independent growth. *Am J Pathol* **168**, 1345-1353, doi:10.2353/ajpath.2006.050596 (2006).

- Human NSCLC cell lines and NIH-3T3 (mouse fibroblast cell line)
- 7/10 NSCLC cell lines expressed AQP1 with 60%+ overexpression
- Forced expression of AQP1 in NIH-3T3 induces proliferation (?)
- No gene amplification or mutation of AQP1 whatsoever in NSCLC cell lines

- Fos/Jun may bind to AQP1 promoter

9 Tomita, Y. *et al.* Role of Aquaporin 1 Signalling in Cancer Development and Progression. *Int J Mol Sci* **18**, doi:10.3390/ijms18020299 (2017).

- AQP1 modulates migration/invasion by either: increase in osmolality due to actin disassembly; or 'osmotic engine'
- Increase in angiogenesis (mechanism unknown)
- Upstream I: cAMP/PKA (?)
- Upstream II: HIF-1a (with glycolysis; observed in rat glioma cells) binds to promoter
- Downstream I: inhibits b-catenin degradation – up proliferation; down differentiation and apoptosis
- Downstream II: upregulates FAK – EMT up, resulting in invasion

HIF1a	0	1	2
AQP1	0**/1**	1**/2**	2**/1**
b-catenin	0**/1**	1**/2**	2**/1**
FAK	**1**	**2**	**1**

10 Machida, Y. *et al.* Relationship of aquaporin 1, 3, and 5 expression in lung cancer cells to cellular differentiation, invasive growth, and metastasis potential. *Hum Pathol* **42**, 669-678, doi:10.1016/j.humpath.2010.07.022 (2011).

- Human NSCLC tissue sample
- Non-neoplastic lung tissue: variable, localised expression of AQP1,3,5; AQP1 expressed in proliferating type II pneumocytes
- Tumour cells: 71%, 40%, 56% positive of AQP1,3,5 respectively
- Lower differentiation associated with lower %cells expressing AQPs (**not significant**)
- Invasive adenocarcinoma: stronger, non-localised expression of AQP1/5
- Lack of correlation between AQP1/5 overexpression and proliferation/p53 status

Differentiation	1	0
Invasion	2	1
AQP1/5	2	1

11 Jablonski, E. M. *et al.* Decreased aquaporin expression leads to increased resistance to apoptosis in hepatocellular carcinoma. *Cancer Lett* **250**, 36-46, doi:10.1016/j.canlet.2006.09.013 (2007).

- Rat hepatoma cell line
- cAMP/PKA upregulates AQP8/9, resulting in apoptotic volume decrease (AVD) hence apoptosis

- Decreased expression, possibly due to inhibitory G proteins (Gi), increases resistance to apoptotic stimuli (e.g. TGF-b)

AQP8/9	0	1	2	
TGF-b	1	1	1	(hypoxia=2 OK)
Apoptosis	0	1	2	
Gi	2	1	0	

12 Thiagarajah, J. R., Chang, J., Goettel, J. A., Verkman, A. S. & Lencer, W. I. Aquaporin-3 mediates hydrogen peroxide-dependent responses to environmental stress in colonic epithelia. *Proc Natl Acad Sci U S A* **114**, 568-573, doi:10.1073/pnas.1612921114 (2017).

- Caco-2 (colon adenocarcinoma) cell line transfected with AQP3-KD showed decreased H_2O_2 uptake and rate in wound healing assay, and defective lamellipodia
- AQP3 knocked out mice showed slower healing
- ***NB*** based on Liu & Bodmer (PNAS, 2005), Caco-2 has no p53 protein detected and a mutated TP53 gene
- Possibly via FAK pathway (as in Kusayama *et al.*, 2011)

AQP3	2(basal)	**1**	
Migration	2	1	
Proliferation	1	0	(if p53 OFF)
Apoptosis	0	0	

13 Satooka, H. & Hara-Chikuma, M. Aquaporin-3 Controls Breast Cancer Cell Migration by Regulating Hydrogen Peroxide Transport and Its Downstream Cell Signaling. *Mol Cell Biol* **36**, 1206-1218, doi:10.1128/MCB.00971-15 (2016).

- Human breast cancer cell line (DU4475 & MDA-MB-231)
- ***NB*** DU4475: wt p53; MDA-MB-231: mut p53 (migration: p53 ON/OFF)

siAQP3	0	0	1	0	1
CXCL12	0	2	2	2	0
Nox2	\	\	\	0	\
AQP3	\	\	1	\	1
H_2O_2	1	2	0	0	\
Migration	1/2	2	0/1	\	0/1
Akt	1	2	0	\	0

PTP	1	0	2	\	2

14 Hara-Chikuma, M., Watanabe, S. & Satooka, H. Involvement of aquaporin-3 in epidermal growth factor receptor signaling via hydrogen peroxide transport in cancer cells. *Biochem Biophys Res Commun* **471**, 603-609, doi:10.1016/j.bbrc.2016.02.010 (2016).

- A431 (human skin SCC) & H1666 (human lung adenocarcinoma)
- EFGR co-immunoprecipitated with AQP3 (A431)
- PTEN might be downstream of AQP3 mediated intracellular H_2O_2 signalling
- EFGR & Nox2 level not affected by siAQP3

15 Meng, F. *et al.* Aqp1 enhances migration of bone marrow mesenchymal stem cells through regulation of FAK and beta-catenin. *Stem Cells Dev* **23**, 66-75, doi:10.1089/scd.2013.0185 (2014).

- Murine bone-marrow-harvested MSC; scratch assay & *in vivo* fracture induction
- FAK co-immunoprecipitated with AQP1

AQP1	1	0	2	1	2
Migration	1	0	2	0	1
Proliferation			(unchanged)		
FAK	1	0	2	**0**	**0**
β-catenin	1	0	2	\	\

16 Goldstein, I. *et al.* p53 promotes the expression of gluconeogenesis-related genes and enhances hepatic glucose production. *Cancer Metab* **1**, 9, doi:10.1186/2049-3002-1-9 (2013).

- Hep-G2 cell line (well-differentiated human liver cancer)

sh-p53	0	1	0
p53	1	0	**2** (nutlin-3a)
Glucose	1	0/1	2
AQP9/3	1	0	2

17 Shaikh, D. *et al.* cAMP-dependent protein kinase is essential for hypoxia-mediated epithelial-mesenchymal transition, migration, and invasion in lung cancer cells. *Cell Signal* **24**, 2396-2406, doi:10.1016/j.cellsig.2012.08.007 (2012).

- A549 (human lung adenocarcinoma; wt p53) and RLE-6TN (rat alveolar epithelial)

- Elevated PKA activity during hypoxia is ROS/HIF dependent and TGF-β1 independent
- PKA necessary for hypoxia-mediated EMT in A549
- Forskolin did not induce EMT in RLE-6TN under normoxia (conclusion is PKA not sufficient?)
- Inhibition of PKA (by H89) prevented TGF-β1-mediated cell migration
- PKACA inhibition resulted in no change in proliferation rate (A549, normoxia)

HIF-1α	**1**	**2**	\
H89	0	0	**1**
PKA	1	2	0
CREB	2	3	\
Invasion (EMT)	1	0	0

18 Wang, W. & Zheng, M. Role of cAMP-PKA/CREB pathway in regulation of AQP 5 production in rat nasal epithelium. *Rhinology* **49**, 464-469, doi:10.4193/Rhino10.107 (2011).

- Murine nasal epithelial cell, treated with either forskolin or H89

forskolin	1	0	0
H89	0	1	0
AQP5	**2**	**0**	1 (basal)
CREB	**3**	**1**	2 (basal)

19 Jessica Chen, M. *et al.* Water and ion channels: crucial in the initiation and progression of apoptosis in central nervous system? *Curr Neuropharmacol* **6**, 102-116, doi:10.2174/157015908784533879 (2008).

- Neurons treated with lactacystin (inducing apoptosis)
- First AQP4 -50% + AQP8/9 increased dramatically, then returned to normal
- cAMP level is bell-shaped as well (Fenteany & Schreiber, 1998)
- High cAMP delayed apoptosis (Insel *et al.*, 2012)

cAMP	1(basal)	2
AQP8/9	1	2
AQP4	2	1

20 Yang, F., Kawedia, J. D. & Menon, A. G. Cyclic AMP regulates aquaporin 5 expression at both transcriptional and post-transcriptional levels through a protein kinase A pathway. *J Biol Chem* **278**, 32173-32180, doi:10.1074/jbc.M305149200 (2003).

- MLE-12 (murine lung epithelial) *in vitro*
- Cpt-cAMP (selective PKA activator) or forskolin addition resulted in 4-fold transient increase in AQP5 and translocation to membrane (i.e. time-dependent)
- H89 or actinomycin D abolished the increase

cAMP	1	1	2	2
H89	0	1	0	1
PKA	\	0	\	0
AQP5	1	**0**	2	**0**

21 Herrlich, A., Leitch, V. & King, L. S. Role of proneuregulin 1 cleavage and human epidermal growth factor receptor activation in hypertonic aquaporin induction. *Proc Natl Acad Sci U S A* **101**, 15799-15804, doi:10.1073/pnas.0406853101 (2004).

- MLE-12 (human lung epithelial) & Calu-3 (**human lung adenocarcinoma**)
- Hypertonocity activates HER2/3 complex and subsequent ERK signalling
- MLE-12: hypertonicity led to AQP5 expression; inhibition of HER2/3 partially reversed the increase; but inhibition of MMP completely abolished increase
- Calu-3: AQP5 already expressed; hypertonicity increases AQP5 and inhibition of HER2/3 reversed the increase (therefore high level of RTK is only sufficient)

RTK	2	1/0
AQP5	X+1	X