

Aquaporin 1 suppresses apoptosis and affects prognosis in esophageal squamous cell carcinoma

SUPPLEMENTARY MATERIALS

Supplementary Table 1A: The 20 genes that displayed the most up-regulated change in their expression levels in the AQP1 siRNA transfected TE5 cells

Symbol	Gene Name	UniGene ID	Expr Fold Change
TRPC7	Transient receptor potential cation channel subfamily C member 7	Hs.591263	176.947
FCRL3	Fc receptor like 3	Hs.292449	128.186
GPC5	Glypican 5	Hs.655675	100.456
OR6C1	Olfactory receptor family 6 subfamily C member 1	Hs.553763	83.208
FGF1	Fibroblast growth factor 1	Hs.483635	79.713
CBR1	Carbonyl reductase 1	Hs.88778	79.535
BOD1L2	Biorientation of chromosomes in cell division 1 like 2	Hs.127882	73.096
THPO	Thrombopoietin	Hs.1166	65.493
IRF7	Interferon regulatory factor 7	Hs.166120	58.113
MYOT	Myotilin	Hs.84665	56.299
GABBR1	Gamma-aminobutyric acid type B receptor subunit 1	Hs.167017	51.456
NXF5	nuclear RNA export factor 5	Hs.307077	46.64
GSTM2	Glutathione S-transferase mu 2	Hs.279837	44.351
NME9	NME/NM23 family member 9	Hs.660992	42.84
MS4A4A	Membrane spanning 4-domains A4A	Hs.325960	42.187
TMEM132B	Transmembrane protein 132B	Hs.524838	42.053
TAS2R13	Taste 2 receptor member 13	Hs.679407	39.57
ETFBKMT	Electron transfer flavoprotein beta subunit lysine methyltransferase	Hs.740628	38.782
SERPINC1	Serpin family C member 1	Hs.75599	37.255
ABCA8	ATP binding cassette subfamily A member 8	Hs.58351	34.346

Supplementary Table 1B: The 20 genes that displayed the most down-regulated change in their expression levels in the AQP1 siRNA transfected TE5 cells

Symbol	Gene Name	UniGene ID	Expr Fold Change
PKD2L2	Polycystin 2 like 2, transient receptor potential cation channel	Hs.716884	-138.177
FAM196B	Family with sequence similarity 196 member B	Hs.721917	-127.583
MCMD2C2	Minichromosome maintenance domain containing 2	Hs.677620	-100.672
CCR2	C-C motif chemokine receptor 2	Hs.132310	-100.025
ABC5	ATP binding cassette subfamily B member 5	Hs.437006	-94.806
BARHL2	BarH like homeobox 2	Hs.511794	-93.257
MAGEE2	MAGE family member E2	Hs.404102	-93.173
OSBPL6	Oxysterol binding protein like 6	Hs.451956	-84.326
AATK	Apoptosis associated tyrosine kinase	Hs.356869	-82.49
TEX12	Testis expressed 12	Hs.318775	-82.283
NDNF	Neuron derived neurotrophic factor	Hs.514575	-80.469
CSDC2	Cold shock domain containing C2	Hs.524039	-76.808
PARP15	Poly(ADP-ribose) polymerase family member 15	Hs.709520	-73.573
PROK2	Prokineticin 2	Hs.310893	-73.559
CPO	Carboxypeptidase O	Hs.120250	-72.393
CCDC127	Coiled-coil domain containing 127	Hs.528665	-71.182
TTL9	Tubulin tyrosine ligase like 9	Hs.684103	-70.434
MEOX1	Mesenchyme homeobox 1	Hs.293833	-69.256
COL13A1	Collagen type XIII alpha 1 chain	Hs.712915	-66.308
OR10Q1	Olfactory receptor family 10 subfamily Q	Hs.438	-65.996

Supplementary Table 2: Top biological functions and canonical pathways of AQP1 according to an Ingenuity Pathway Analysis

Top biological functions		
Diseases and disorders		
Name	P value	Number of molecules
Cancer	5.74E-04–9.13E-12	3515
Organismal injury and abnormalities	5.74E-04–9.13E-12	3594
Reproductive system disease	5.74E-04–9.13E-12	1763
Inflammatory response	5.55E-04–9.46E-12	797
Dermatological diseases and conditions	9.81E-05–5.55E-09	233
Molecular and cellular functions		
Name	P value	Number of molecules
Cellular movement	5.20E-04–1.14E-09	817
Cellular development	5.88E-04–4.14E-09	953
Cellular growth and proliferation	5.88E-04–4.14E-09	972
Cell morphology	5.29E-04–1.42E-08	932
Cellular assembly and organization	2.55E-04–1.42E-08	551
Top canonical pathways		
Name	P value	Number of molecules
Dendritic cell maturation	7.37E-07	37.8% (65/172)
Communication between innate and adaptive immune cells	2.37E-06	46.5% (33/71)
Antigen presentation pathway	3.07E-06	56.8% (21/37)
Graft-versus-host disease signaling	2.41E-05	52.6% (20/38)
Role of pattern recognition receptors in recognition of bacteria and viruses	3.15E-05	37.2% (48/129)

Supplementary Table 3: Backgrounds of 50 ESCC patients

Sex		
Male	42	84%
Female	8	16%
Age		
average	62.4	
<65	30	60%
≥65	20	40%
Smoking (BI)		
Non smoking	10	20%
<1200	14	28%
≥1200	16	32%
N.A	10	20%
Drinking (ethanol: g/day)		
Non drinking	14	28%
Opportunity	5	10%
drink	17	34%
<60	13	26%
≥60	1	2%
N.A		
Histology type		
Well/Moderate	35	70%
Poor	15	30%
Location		
Ce-Ut	5	10%
Mt	24	48%
Lt-Ae	21	42%
Tumor size (mm)		
<50	31	62%
≥50	19	38%
Lymphatic invasion		
Negative	22	44%
Positive	28	56%
Venous invasion		
Negative	26	52%
Positive	24	48%
pT		
pT1	19	38%
pT2-4	31	62%
pN		
pN0	19	38%
pN1-3	31	62%
pStage		
1	9	18%
2	25	50%
3	13	26%
4	3	6%
Postoperative therapy		
Chemotherapy	31	62%
Radiation	11	22%
Reccurrence		
Positive	20	40%
Negative	30	60%

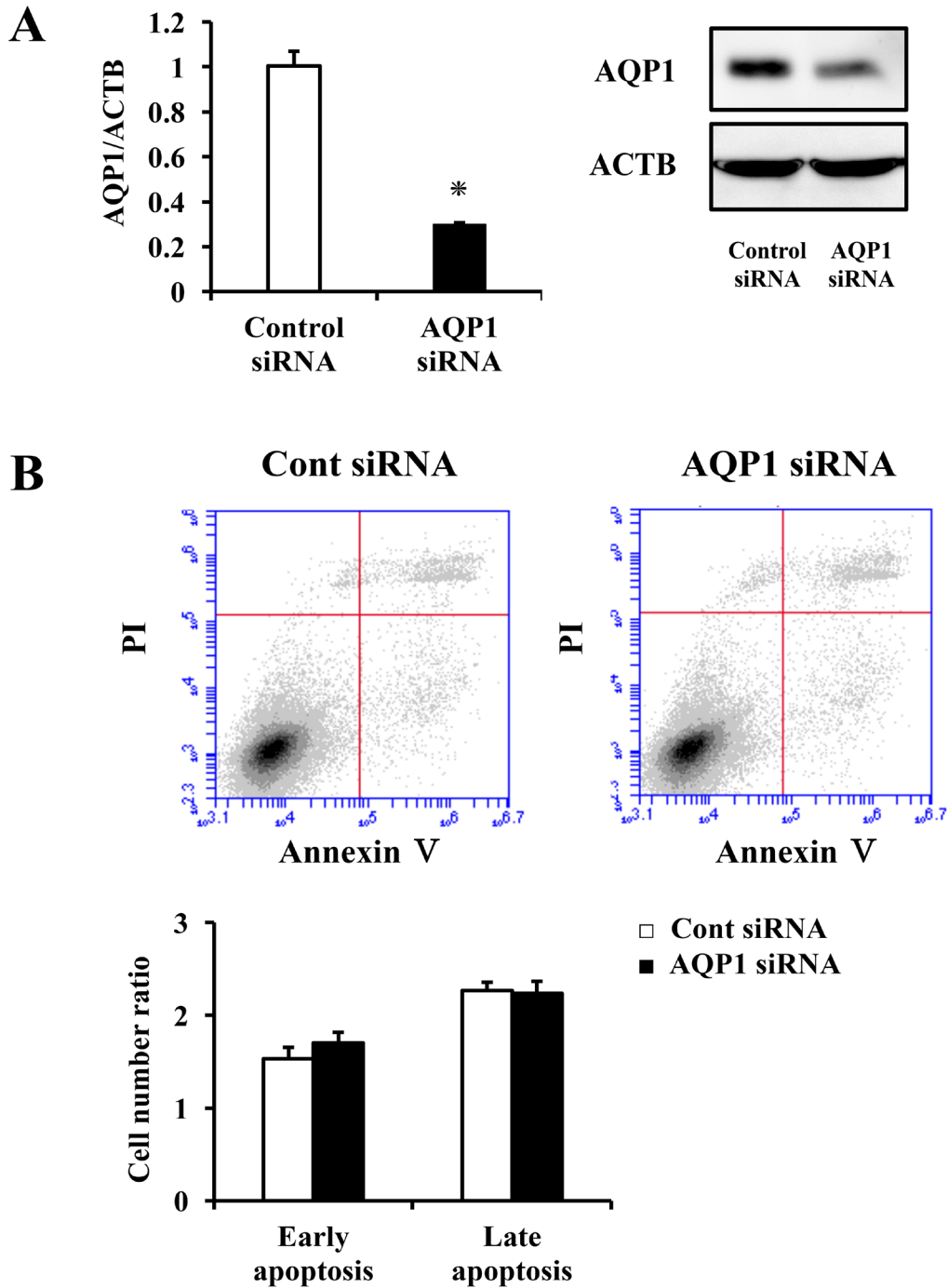
BI:Brinkmann index

pT: pathological T stage, pN: pathological N stage

* $p < 0.05$: Fisher's exact test.

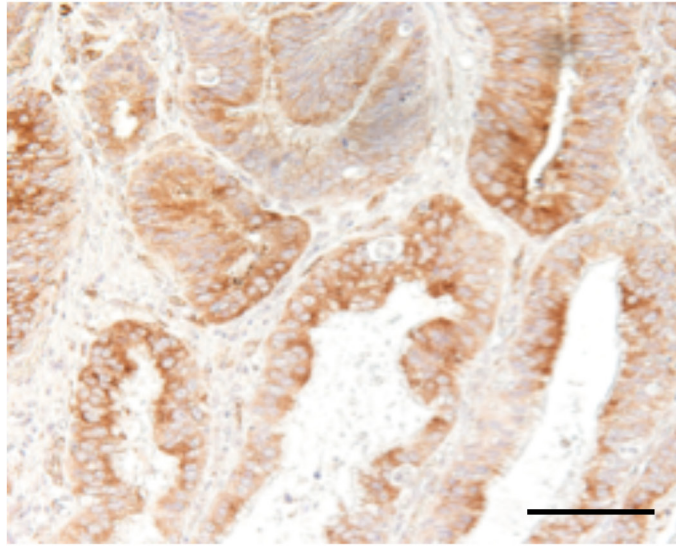
Supplementary Table 4: 5-year overall survival rate with each cut-off values for cytoplasm and nuclear membrane respectively

Population	<i>Cytoplasm</i>			<i>Nuclear membrane</i>		
	5-year overall survival rate		<i>P</i> value	5-year overall survival rate		<i>P</i> value
	Low group	High group		Low group	High group	
10	81.2% (n=16)	62.8% (n=34)	0.235	62.7% (n=19)	72.7% (n=31)	0.495
20	76.0% (n=25)	61.5% (n=25)	0.305	63.6% (n=28)	75.5% (n=22)	0.457
30	70.3% (n=31)	66.5% (n=19)	0.996	61.0% (n=35)	86.6% (n=15)	0.121
40	70.8% (n=35)	65.0% (n=15)	0.919	63.6% (n=40)	90.0% (n=10)	0.168

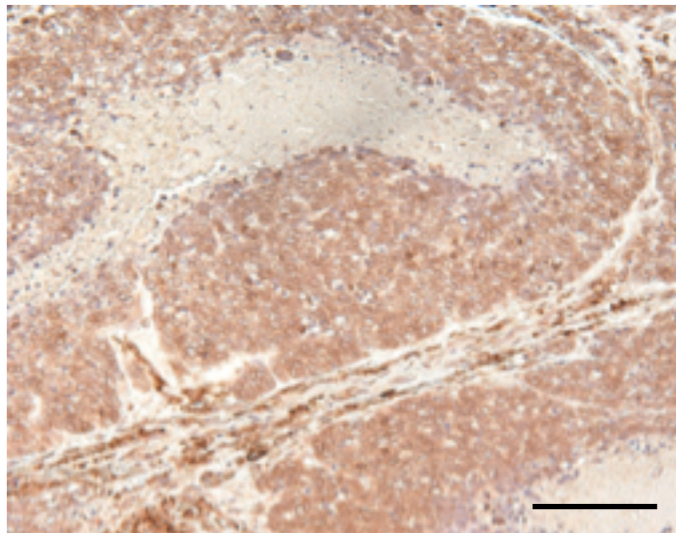


Supplementary Figure 1: AQP1 has no regulation of the cell survival in KYSE70 cells. (A) AQP1 siRNA effectively reduced AQP1 mRNA levels in KYSE70 cells. Mean \pm SEM. $n = 3$. * $p < 0.05$ (significantly different from control siRNA). Western blotting revealed that AQP1 siRNA effectively reduced AQP1 protein levels in KYSE70 cells. (B) AQP1 had no influence on apoptosis in KYSE70 cells. Apoptosis was determined by flow cytometry using PI/Annexin V double staining. Mean \pm SEM. $n = 3$. * $p < 0.05$ (significantly different from control siRNA).

A *Esophageal adenocarcinoma*



B *Esophageal basaloid cell carcinoma*



Supplementary Figure 2: AQP1 protein expression in human esophageal adenocarcinoma and esophageal basaloid-type SCC. (A) Immunohistochemical staining of human esophageal adenocarcinoma using an anti-AQP1 antibody. AQP1 was expressed in esophageal adenocarcinoma. Magnification: $\times 200$. Bar 100 μm . **(B)** Immunohistochemical staining of human esophageal basaloid-type SCC using an anti-AQP1 antibody. AQP1 was expressed in esophageal basaloid-type SCC. Magnification: $\times 200$. Bar 100 μm .