

SUPPLEMENTARY METHODS

A novel long non-coding RNA regulates the integrin, ITGA2 in breast cancer

Breast Cancer Research and Treatment

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Methods

Cell Culture Conditions. MDA-MB-231 and MDA-MB-453 cells were cultured in L-15 Medium Leibovitz (Sigma-Aldrich) and incubated in atmospheric CO₂ at 37 °C. MCF-7 cells were cultured in Minimum Essential Medium Eagle (Sigma-Aldrich) and T-47D in RPMI 1640 Media (ThermoFisher Scientific), both incubated in 5% CO₂ at 37 °C. Media was supplemented with 10% foetal calf serum and 2% penicillin-streptomycin.

Supplementary Table 1: Characteristics of BrCa and PrCa cell lines used in this study.

Cell line	Cell Type	Marker Status	Cell line Characteristics
MDA-MB-231	Invasive Basal cell Adenocarcinoma derived from metastasis (PE) (Claudin-low)	ER ⁻ / PR ⁻ / HER2 ⁻	Tumourigenic Aggressive metastatic
MDA-MB-453	Invasive Basal cell Adenocarcinoma from metastasis (PE)	ER ⁻ / PR ⁻ / HER2 ⁺	Non-tumourigenic Non metastatic
T-47D	Luminal A Invasive ductal carcinoma from metastasis (PE)	ER ⁺ / PR ⁺ / HER2 ⁻	Tumourigenic Non-metastatic
MCF-7	Luminal A Invasive ductal carcinoma from metastasis (PE)	ER ⁺ / PR ⁺ / HER2 ⁻	Tumourigenic Non/weakly metastatic
PC3	adenocarcinoma from bone metastasis.	AR ⁻	Tumourigenic / Aggressive metastatic
LNCaP	adenocarcinoma from lymph node metastasis	AR ⁺	Tumourigenic / Non/weakly metastatic

ER = estrogen receptor alpha, PR = progesterone receptor, HER2 = human epidermal growth factor receptor 2, AR= androgen receptor, PE=pleural effusion.

References

Maemura, M., *et al.* (1995) Expression and ligand binding of $\alpha 2\beta 1$ integrin on breast carcinoma cells. *Clinical & Experimental Metastasis*, **13**, 223–235.

Chin, S.P., *et al.* (2015) Regulation of the *ITGA2* Gene by Epigenetic Mechanisms in Prostate Cancer. *The Prostate*, **75**, 723–734.

Dai, X., *et al.* (2017) Breast cancer cell line classification and its relevance with breast tumor subtyping. *Journal of Cancer*, **8**, 3131–3141.

Cailleau, R., *et al.* (1978) Long-term human breast carcinoma cell lines of metastatic origin: preliminary characterization. *In Vitro*, **14**, 911–915.

Supplementary Methods Table 2. Primers used for PCR and qPCR reactions.

Primer	Sequence (5' – 3')	Amplicon size (bp)	qPCR / PCR annealing conditions (°C)
<i>hGAPDH</i> Forward	5'- AAATATGATGACATCAAGAAGG	67	60
<i>hGAPDH</i> Reverse	5'- AGCCCAGGATGCCCTTGAGGG		
<i>ITGA2</i> Forward	5' – CTCACCAGGAACATGGGAAC	99	60
<i>ITGA2</i> Reverse	5' - GTCAGAACACACACCCGTTG		
<i>I2ALR</i> set 2 Forward	5'- AGGTACAAGGCCTGAAAGAACC	74	62
<i>I2ALR</i> set 2 Reverse	5'- TTTAGAACACCGCTGGCAGG		
<i>I2ALR</i> set 5 Forward	5'- GCTTCGTCCTCAAATCTTCATGG	84	60
<i>I2ALR</i> set 5 Reverse	5'- TGAGCCACTTTGCTTCCTAGAG		
<i>I2ALR</i> 3'-end set 1 Forward	5'- AGCTTCCACAGGATGAGAACG	507*	59
<i>I2ALR</i> 3'-end set 1 Reverse	5'- ATGGTTTGTCTGAATGTGCCG		
<i>I2ALR</i> 3'-end set 2 Forward	5'- CCTGCCAGCGGTGTTCTAA	610*	59
<i>I2ALR</i> 3'-end set 2 Reverse	5'- GCCAGGATAAGCTTCTCTTTCAAAC		
<i>TWIST1</i> Forward	5'- GAGAGATGATGCAGGACGTG	72	60
<i>TWIST1</i> Reverse	5'- CTTCTCGCTGTTGCTCAG		
<i>CCND1</i> Forward	5'- GCTGCGAAGTGGAACCATC	135	60
<i>CCND1</i> Reverse	5'- CCTCCTTCTGCACACATTTGAA		
<i>ACLY</i> Forward	5'- ATCGGTTCAAGTATGCTCGGG	132	60
<i>ACLY</i> Reverse	5'- GACCAAGTTTTCCACGACGTT		

Supplementary Methods Table 3. Primers for ITGA2 Bisulphite Converted DNA

ITGA2 promoter fragment	Sequence	Amplicon size (bp)
BSITGA1F Forward	5' – TTGATGAGTTAGTTTTTAATTTGG	120
BSITGA1R Reverse	5' – AACCCCTAAACCACAATACCTAA	
BSTITGA2F Forward	5' – TTAGGTATTGTGGTTTAGGGTT	429
BS46R Reverse	5' – AAAAATTCTAAACAACCTCCTACA	

Supplementary Methods Table 4. ASO gapmers used for knockdown of *I2ALR*, with chemical modifications and additional information provided by supplier. * ‘^’ = Phosphorothioated (PS) backbone, underlined = 2’-O-methoxy-ethyl (2’-O-MOE, or 2’-MOE) RNA (non-underlined = DNA bases).

Name	Sequence*	Molecular weight (g.mol ⁻¹)	Extinction coefficient (L/(mole.cm))	Amount mg (nmoles)
ASO-160	5’ <u>A^A^G^A^G^A^C^A^T^G^A^T^G^A^T^A^T^A^C^A^T^A^T^A^C^A^C^A^G</u>	7177	177 800	0.85 (118.5)
ASO-991	5’ <u>T^A^T^A^G^A^T^A^C^A^T^A^G^A^A^A^A^T^A^G^A^T^G^A^C^A^C^A^G^A^T^A^G^A^T^A^A</u>	7214	195 700	0.81 (112.6)
ASO-Scr scrambled	5’ <u>G^A^C^A^G^A^A^C^A^T^A^A^T^A^A^C^A^G^A^C^A^C^A^A^A^A^T^A^A^T^A^G</u>	7200	199 600	0.91 (125.6)

Supplementary Methods Table 5. *I2ALR*^{construct} sequence for over-expression, showing the insert sequence and flanking *NheI* and *XhoI* restriction enzyme sites (green and blue respectively). Alternating exons are coloured red and grey, poly-A signal and cut signal motif highlighted red, and poly-A highlighted pink. The uncharacterized 3’ region (U3R) is highlighted grey.

Sequence		
GCTAGGATTGCGGCATCTAGGATCCAGAAGTGAAAAGAACTTTTCCTAGCCTAAGTTTACATTTCGTCG AATAGACAATGTTACAACAATAAGAGGAAGGCAGAAGGGAAGGAAGAATCCCCCTCGCCAAGTATT TAAGTGGCGCTTGGTTTATTCCAAGTCCCCCGGGAAGAAACACAGCGCTTCGTCCTCAAATCTTCAT GGAATGCTACTATGAGCTGAATGGGATGCCTGAGATACTGCCTCTAGGAAGCAAAGTGGCTCAAGAG GCTGGCTGGCAAGATGGCCGAATAGGAACAGCTCCAGTCTGCAGCTCCTAGTGAGATCAACGCCAAA GGCGGGTGACTTCTGCATTTCCTCACTGAGATACCCAGCTCATCTCACTGGGACTGGTTAGACAGTGGG TGCAGCCCATGAGGTACAAGGCCTGAAAGAACCTATCCAAATATCAGCTCTGGTTTTCTCTCTCCTG CCAGCGGTGTTCTAAACCATAGAGATTGAGAAGACTGATCAGCTTCCACAGGATGAGAACGATGAC CAAGAAATATGCTGGCGGCCTCTAAGAAAATATCGTGTTTTGCGAAGGACTTCTGGGTCTTCAACTTG CATTTCGGCAATTAAGACTGGACAGCAAATGCAATCCGGTATCACTAACACCTAACACTTTGGGTTATT TAGTATAATGGTTCTCAAACATTAGGATGCATGAGAATTACCTGGAAGGCTTGTTAAACCCATCCTC CGAGTTTCTGATTTGGTAGGTTTCTGGTGGGGCCAGAGAATTTGCCTAACAAGTTTCCAGGTGATGCT GATGCCCTTGGCCAGGCAGGGACCACACTTTCAGAACCACTGGGTTAATAGAAAGGCATCCACATAA AAATGTAGTTTTCTCTACCTGTAAACTGTAGTCCAACCTTCATATTTTACCGGGATAGAAATGTTTAC TACATTATCAGAAAGGGTTTCAGGAGGTTCTTCGCTGTCACTAGAAAATACACGGCACATTCAGACAA ACCATTAATAATCTTGACTATTTAAAGACATTTCTAAGTGTTTGAAAGAGAAGCTTATCCTGGCAACTA AATAAAAGCATGTAAGAGTATAATTTTAAATCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAA AAACTCGAG-3’		
Base pair composition	GC composition	Base pair length
A = 440, T = 295, C = 240, G = 257	40.34%	1232 (1222 following restriction enzyme digest)