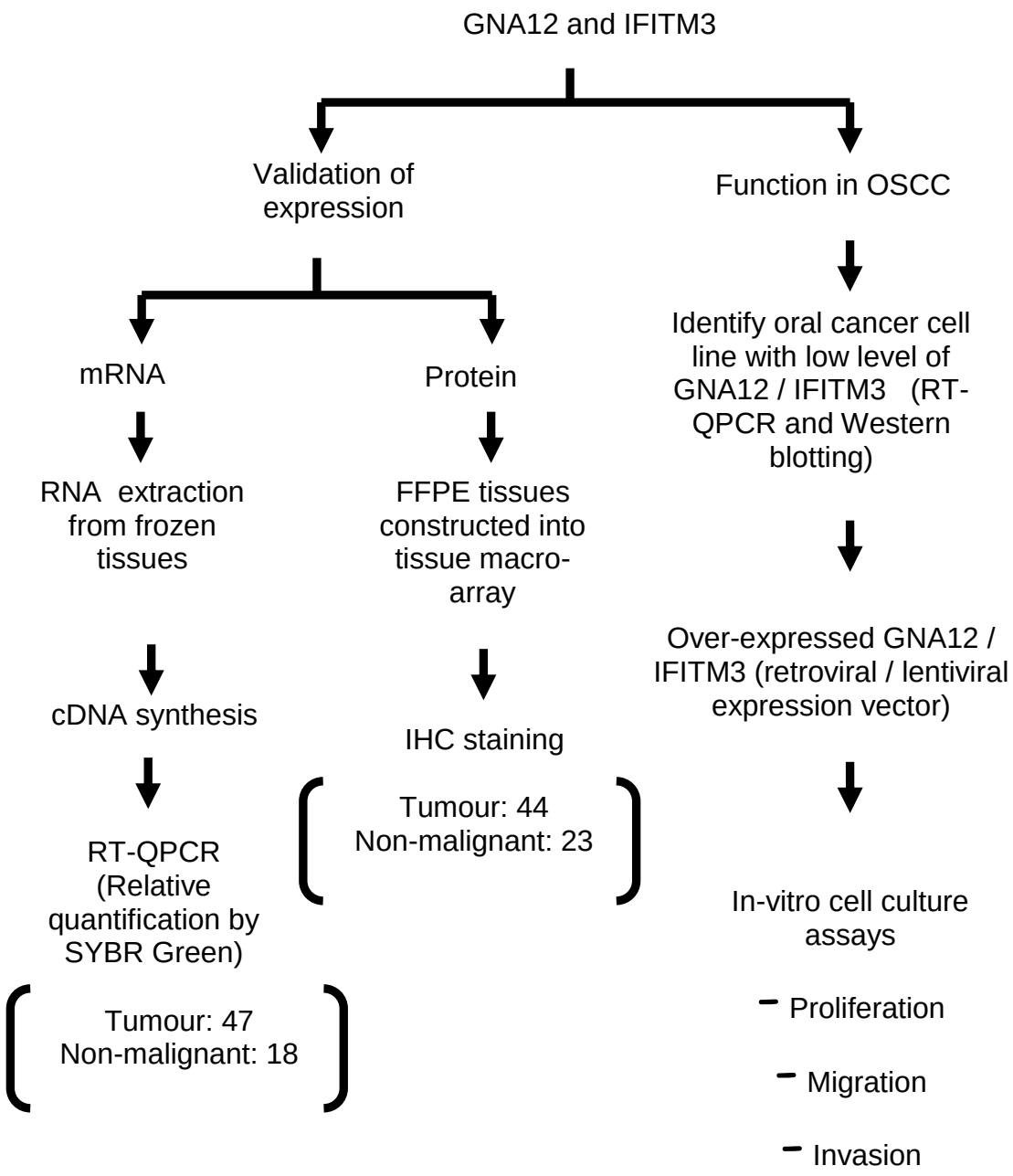
Appendix I

Project Work flow



Appendix II

Frozen tissues specimens used in mRNA QPCR analysis

No	Sample	Type	Age	Ethnic	Sex	Diagnosis	Habits	Site	Broder's grade	TNM
1	01-0002-04	NM	80	Indian	F	SCC	BQ	gum	NA	NA
2	01-0006-04	NM	60	Indian	F	SCC	BQ	cheek	NA	NA
3	01-0017-05-3	NM	24	Malay	M	Impacted 8	No Habit	gum	NA	NA
4	01-0018-05-3	NM	25	Malay	M	Impacted 8	UK	gum	NA	NA
5	01-0020-05	NM	23	Malay	F	Impacted 8	No Habit	gum	NA	NA
6	01-0007-07-3 (1)	NM	35	Malay	M	Impacted 8	Smoke	gum	NA	NA
7	01-0001-08-3	NM	21	Chinese	M	Impacted 8	No Habit	gum	NA	NA
8	01-0004-08-3 (1)	NM	28	Malay	M	Impacted 8	Smoke	gum	NA	NA
9	01-0009-08-3 (1)	NM	27	Malay	M	Impacted 8	No Habit	gum	NA	NA
10	01-0011-08-3 (1)	NM	29	Malay	M	Impacted 8	Smoke	gum	NA	NA
11	01-0018-08-3 (1)	NM	30	Malay	M	Impacted 8	Smoke	gum	NA	NA
12	01-0025-08-3 (1)	NM	23	Chinese	M	Impacted 8	No Habit	gum	NA	NA
13	04-0012-04	NM	39	Chinese	F	SCC	Smoke	tongue	NA	NA
14	04-0001-05	NM	75	Malay	F	SCC	BQ	cheek	NA	NA
15	04-0003-05	NM	52	Malay	M	SCC	Smoke	cheek	NA	NA
16	06-0033-04	NM	70	Indian	F	SCC	BQ	cheek	NA	NA
17	06-0002-05	NM	65	Indian	F	SCC	BQ	cheek	NA	NA
18	AHN	NM	55	Malay	M	SCC	Smoke	gum	NA	NA
19	01-0002-04	Tumour	80	Indian	F	SCC	BQ	gum	Well	T4N1Mx
20	01-0005-04	Tumour	55	Malay	M	SCC	Smoke, Alcohol	palate	Well	T4N0M0
21	01-0006-04	Tumour	60	Indian	F	SCC	BQ	cheek	Well	T4N3M0
22	01-0017-04	Tumour	74	Indian	F	SCC	BQ	tongue	Moderate	T1NxMx
23	01-0005-05 (1)	Tumour	55	Malay	M	SCC	Smoke	maxilla	Well	T2N0M0
24	01-0022-05	Tumour	59	Indian	F	SCC	BQ	cheek	Moderate	T4NxMx
25	01-0038-05	Tumour	52	Chinese	M	SCC	No Habit	tongue	Poor	T3N0Mx
26	01-0046-05	Tumour	45	Chinese	M	SCC	Alcohol	tongue	Moderate	T4N1M1
27	01-0001-06	Tumour	71	Indian	F	SCC	BQ	gum	Well	T3N2bM0
28	01-0005-06	Tumour	58	Indian	F	SCC	BQ	gum	Well	T4N0Mx
29	01-0010-06	Tumour	39	Malay	F	SCC	No Habit	tongue	UK	T4N1Mx
30	01-0029-06 (t2)	Tumour	66	Indian	F	SCC	BQ	mandible	Well	T4NoMo
31	01-0002-07-1 (1)	Tumour	56	Indian	F	SCC	BQ	gum	Well	T4N3M0
32	01-0022-07 (t1)	Tumour	49	Malay	M	SCC	Smoke	palate	Well	T4NxMx
33	01-0024-07-1 (t2)	Tumour	48	Malay	M	SCC	No Habit	tongue	Poor	T2N0Mx
34	01-0040-07-1	Tumour	73	Indian	M	SCC	Smoke, Alcohol	cheek	Well	UK
35	01-0017-08-1 (3)	Tumour	58	Chinese	M	SCC	Alcohol	cheek	UK	T4N2bMx
36	02-0004-04	Tumour	60	Chinese	M	SCC	Smoke, Alcohol	FOM	Poor	T4N2cM0
37	02-0005-05	Tumour	60	Malay	M	SCC	No Habit	cheek	UK	UK
38	02-0018-05	Tumour	37	Malay	M	SCC	Smoke	cheek	Well	T4N2M0
39	03-0004-04	Tumour	56	Malay	F	SCC	UK	UK	UK	UK
40	04-0004-04	Tumour	53	Chinese	M	SCC	Smoke, Alcohol	cheek	Well	T3NxM0
41	04-0005-04	Tumour	70	Indian	F	SCC	BQ, Alcohol	cheek	Well	T2N2M0
42	04-0012-04	Tumour	39	Chinese	F	SCC	Smoke	tongue	Moderate	T2N0M0
43	04-0001-05 (2)	Tumour	75	Malay	F	SCC	BQ	maxilla	Well	T4NoMx
44	04-0003-05	Tumour	52	Malay	M	SCC	Smoke	cheek	Moderate	T4N2bMx
45	04-0017-05	Tumour	66	Malay	F	SCC	No Habit	tongue	Moderate	T2N1M0

Appendix II, continued

Frozen tissues specimens used in mRNA QPCR analysis, continued

46	04-0010-06	Tumour	50	Indian	M	SCC	Smoke	tongue	Moderate	T3N2bM0
47	04-0002-06	Tumour	46	Chinese	M	SCC	No Habit	mandible	Moderate	T3N0Mx
48	04-0006-06	Tumour	71	Indian	F	SCC	BQ	cheek	Well	T4N0Mx
49	04-0021-06-2	Tumour	48	Malay	M	SCC	Smoke	tongue	Moderate	T1N2bMx
50	06-0007-04	Tumour	47	Indian	F	SCC	BQ	cheek	Moderate	UK
51	06-0031-04	Tumour	41	Malay	M	SCC	Smoke	cheek	Moderate	T4NoMx
52	06-0033-04	Tumour	70	Indian	F	SCC	BQ	cheek	Moderate	T4N0Mx
53	06-0045-04	Tumour	48	Indian	F	SCC	Alcohol, BQ	gum	Moderate	T4N2bM0
54	06-0002-05	Tumour	65	Indian	F	SCC	BQ	cheek	Moderate	T2NoMo
55	06-0009-05	Tumour	30	Chinese	F	SCC	No Habit	tongue	UK	T3N0Mx
56	06-0026-05	Tumour	71	Indian	M	SCC	Alcohol	tongue	Moderate	T1N0M0
57	06-0027-05	Tumour	36	Indian	M	SCC	Smoke	palate	Moderate	T4N2bM0
58	06-0039-05	Tumour	28	Indian	F	SCC	No Habit	tongue	Well	T1NoMx
59	06-0067-05	Tumour	56	Malay	M	SCC	Smoke	tongue	Moderate	T3N1Mx
60	06-0009-06	Tumour	61	others	F	SCC	BQ	cheek	Well	T4NoMx
61	11-0005-05	Tumour	67	Malay	M	SCC	No Habit	gum	Well	T4N0Mx
62	11-0031-05	Tumour	57	Malay	F	SCC	BQ	cheek	Moderate	T4N0Mx
63	11-0013-06	Tumour	43	Bidayuh	M	SCC	Smoke, Alcohol	tongue	Well	T3N2aMx
64	11-0014-06	Tumour	59	Iban	F	SCC	No Habit	palate	Moderate	T4N2bMx
65	11-0017-06	Tumour	58	Iban	F	SCC	Alcohol, BQ	gum	Moderate	T3N2aMx
66	11-0005-07-1	Tumour	34	Chinese	M	SCC	Smoke	tongue	Well	T2N1Mx

Note: BQ: betel quid, F: female, FOM: floor of mouth, M: male, NA: not applicable, NM: non-malignant, SCC: squamous cell carcinoma, UK: unknown

Appendix III

TMaA specimens used for IHC staining

No	Case No	Tissue type	IRPA	Age	Sex	Ethnic	Habit	Site	Broder's Grading	TNM
1	OC(S)2	NM	UK	UK	UK	UK	UK	NA	NA	NA
2	D91/92	FEP	UK	45	F	Indian	UK	gum	NA	NA
3	OC(S)6	NM	UK	UK	UK	UK	UK	UK	NA	NA
4	D173/97	FEP	UK	50	M	Indian	UK	gum	NA	NA
5	OC(S)4	NM	UK	UK	UK	UK	UK	NA	NA	NA
6	D30/94	FEP	UK	59	F	Chinese	UK	gum	NA	NA
7	OC(S)3	NM	UK	UK	UK	UK	UK	UK	NA	NA
8	D405/05	FEP	UK	61	F	Chinese	UK	gum	NA	NA
9	OC(S)1	NM	UK	UK	UK	UK	UK	UK	NA	NA
10	D167/04	FEP	UK	52	F	UK	UK	gum	NA	NA
11	OC(S)8	NM	UK	UK	UK	UK	UK	UK	NA	NA
12	D128/06	FEP	UK	46	F	Chinese	UK	gum	NA	NA
13	OC(S)5	NM	UK	UK	UK	UK	UK	UK	NA	NA
14	OC(S)10	NM	UK	UK	UK	UK	UK	UK	NA	NA
15	D533/05	FEP	UK	81	M	Indian	UK	gum	NA	NA
16	D49/87	FEP	UK	44	M	Chinese	UK	gum	NA	NA
17	D70/81	FEP	UK	29	F	M	UK	gum	NA	NA
18	D20/81	FEP	UK	34	F	M	UK	gum	NA	NA
19	3N-1	NM	UK	UK	UK	UK	UK	UK	NA	NA
20	3N-2	NM	UK	UK	UK	UK	UK	UK	NA	NA
21	3N-3	NM	UK	UK	UK	UK	UK	UK	NA	NA
22	3N-D2	NM	UK	UK	UK	UK	UK	UK	NA	NA
23	3N-D3	NM	UK	UK	UK	UK	UK	UK	NA	NA
24	D556/05	SCC	01-0051-05	62	F	Indian	BQ, alcohol	cheek	Well	T4N2aMx
25	D366/06	SCC	06-0045-06	67	F	Indian	BQ, alcohol	cheek	Well	T1N2bMx
26	D9/06	SCC	01-0050-05	73	F	Indian	BQ	cheek	Moderate	T2N0Mx
27	D406/04	SCC	06-0005-04	60	F	Chinese	No habit	cheek	Moderate	T2N0Mx
28	D303/05	SCC	06-0028-05	67	F	Indian	BQ	cheek	Well	T4N0Mx
29	D119/06	SCC	06-0009-06	61	F	Others	BQ	cheek	Moderate	T2N0Mx
30	D48/06	SCC	06-0001-06	59	F	Indian	BQ, alcohol	cheek	Moderate	T2N2bMx
31	D152/06	SCC	06-0015-06	54	F	Indian	BQ	cheek	NA	T1N0Mx
32	D212/02	SCC	UK	62	F	Chinese	BQ, smoke, alcohol	cheek	NA	UK
33	D48/05	SCC	01-0003-05	50	M	Indian	BQ	cheek	Well	T2N0Mx
34	D62/06	SCC	06-0002-06	52	F	Indian	BQ	cheek	Well	T1N0Mx
35	D84/05	SCC	01-0017-04	74	F	Indian	BQ	cheek	Moderate	T1NxMx
36	D273/06	SCC	06-0037-06	49	F	Indian	BQ	cheek	Moderate	T3N0Mx
37	D251/05	SCC	06-0018-05	66	F	Indian	BQ	cheek	UK	T1N0Mx
38	D517/04	SCC	06-0033-04	70	F	Indian	BQ	cheek	Moderate	T4N0Mx
39	D41/05	SCC	06-0045-04	48	F	Indian	BQ, alcohol	cheek	Moderate	T4N1Mx
40	D359/03	SCC	06-0028-03	55	M	Indian	BQ, smoke	cheek	Moderate	T1N0Mx
41	D137/04	SCC	06-0029-03	63	F	Indian	BQ	cheek	Moderate	T2N2bMx
42	D382/03	SCC	01-0022-05	59	F	Indian	BQ	cheek	Moderate	T1N0Mx
43	D212/05	SCC	01-0022-05	59	F	Indian	BQ	cheek	Moderate	T1N0Mx
44	D68/05	SCC	01-0001-05	65	F	Indian	BQ	cheek	Well	T4N0Mx

Appendix III, continued

TMaA specimens used for IHC staining, continued

45	D139/05	SCC	06-0002-05	65	F	Indian	BQ	cheek	Moderate	T4N0Mx
46	D373/04	SCC	01-0011-04	50	F	Indian	BQ	cheek	Moderate	T2N1Mx
47	D49/07	SCC	06-0005-07	72	F	Indian	BQ	cheek	Moderate	T4N1Mx
48	D21/04	SCC	06-0035-03	62	F	Indian	BQ	cheek	Moderate	T3N1Mx
49	D79/07	SCC	01-0017-07	61	F	Indian	BQ	cheek	Moderate	T4N1Mx
50	D66/06	SCC	11-0006-06	73	M	Chinese	BQ	gum	Moderate	T4N0Mx
51	D135/06	SCC	01-0001-06	71	F	Indian	BQ	gum	Well	T4N2bMx
52	D182/06	SCC	06-0022-06	72	F	Indian	No habit	gum	Well	T3N2bMx
53	D88/05	SCC	01-0005-05	54	M	Malay	smoke	gum	Well	T4N0Mx
54	D498/06	SCC	06-0060-06	58	F	Indian	No habit	gum	Well	T3N2aMx
55	D579/05	SCC	06-0066-05	69	M	Indian	No habit	gum	Well	T1N0Mx
56	D473/05	SCC	06-0040-05	75	F	Indian	BQ	gum	Moderate	T4N2bMx
57	D422/04	SCC	06-0031-04	41	M	Malay	smoke	gum	Moderate	T2N2bMx
58	D38/07	SCC	06-0066-06	71	F	Indian	No habit	gum	Poor	T4N0Mx
59	D146/04	SCC	01-0002-04	80	F	Indian	BQ	gum	Well	T4N0Mx
60	D134/07	SCC	01-0022-07	49	M	Malay	smoke	gum	Well	T4N0Mx
61	D501/05	SCC	06-0051-05	65	M	Indian	smoke, alcohol	FOM	Moderate	T1N2bMx
62	D486/04	SCC	06-0041-04	68	F	Indian	BQ, alcohol	cheek	Poorly	T4N0Mx
63	D241/05	SCC	01-0025-05	38	M	Chinese	smoke, alcohol	tongue	Moderate	T1N2aMx
64	D67/07	SCC	06-0003-07	49	M	Malay	smoke, alcohol	tongue	Well	T1N0Mx
65	D140/05	SCC	06-0011-05	56	M	Indian	BQ, smoke, alcohol	tongue	Poor	T1N0Mx
66	D264/06	SCC	06-0029-06	59	F	Indian	BQ	FOM	Moderate	T1N2bMx
67	D34/06	SCC	06-0067-05	57	M	Malay	smoke	tongue	Moderate	T2N2bMx

Note: BQ: betel quid, F: female, FEP: fibro epithelial polyps, FOM: floor of mouth, M: male, NA: not applicable, SCC: squamous cell carcinoma, UK: unknown

Appendix IV

Cell line information

No	Cell line	Ethnic	Gender	Age	Site	Broder's grade	Pathology staging
1	ORL48	Sikh	Female	79	Gum	Well	T4N2aMx
2	ORL115	Malay	Female	75	Cheek	Well	T2NxMx
3	ORL136	Indian	Male	57	Tongue	Poor	T1N0Mx
4	ORL150	Indian	Male	71	Tongue	Moderate	T1N0Mx
5	ORL153	Indian	Male	36	Maxillary	Moderate	T4N2BMx
6	ORL156	Chinese	Male	38	Tongue	Moderate	T4N1Mx
7	ORL166	Malay	Female	67	Tongue	Moderate	T3N1Mx
8	ORL174	Indian	Female	53	Tongue	Moderate	Unknown
9	ORL188	Malay	Male	57	Tongue	Moderate	T2N0Mx
10	ORL195	Indian	Female	61	Cheek	Well	T4N0Mx
11	ORL196	Indian	Female	49	Cheek	Moderate	T4N1Mx
12	ORL204	Indian	Male	76	Cheek	Moderate	T4N1Mx
13	ORL207	Sikh	Female	59	Tongue	Unknown	Unknown
14	ORL214	Indian	Female	49	Cheek	Moderate	Unknown
15	ORL215	Indian	Male	50	Tongue	Moderate	T3N2bMx
16	ORL218N	Indian	Male	26	Gum	Not applicable	Not applicable
17	ORL232N	Malay	Female	26	Gum	Not applicable	Not applicable

Appendix V

RNA extraction

(A) Frozen tissues

RNA was extracted from cryosectioned tissues using the RNeasy micro kit (Qiagen, Hilden, Germany). Cryosectioned tissues were lysed by vortexing in RLT buffer containing 10 µl/ml β-mercaptoethanol (Sigma, MO, USA). Subsequently, 1 volume of 70% ethanol was mixed to the homogenized lysate and transferred to the spin column for centrifugation at 12, 000rpm for 15 sec. Sample was incubated with 80 µl DNase I at room temperature for 15 min and washed with 0.5 ml RW1 buffer to remove DNA. RNA was washed with 0.5 ml buffer RPE and 0.5 ml 80% ethanol. Finally, the spin column was subjected to full speed centrifugation for 5 min to dry the membrane before eluting the RNA with 20 µl RNase-free water.

(B) Oral cell lines

Oral cancer cells were grown to 70% confluency. The cell monolayer was rinsed with 1x PBS and 1 ml of Tri-Reagent (MRC, OH, USA) was added to every 10 cm^2 of culture dish area. Cells were incubated at room temperature for 5 min to allow dissociation of nucleoprotein complexes. The homogenate was harvested mixed with 0.2 ml of chloroform by vigorous shaking for 15 sec. Following incubation at room temperature for 15 min, the mixture was centrifuged at 12,000 g for 15 min at 4ºC for phase separation. The upper aqueous phase containing the RNA was harvested for RNA precipitation by 0.5 ml of isopropanol for 10 mins at room temperature. Pelleted RNA was washed with 75% ethanol and centrifuged at 7,500 g for 5 mins at 4ºC. Finally, RNA pellet was air-dried and dissolved in 30 µl of RNase-free water.

RNA concentration was quantitated by NanoDrop ND-1000 spectrophotometer, according to manufacturer's instruction. RNase-free water was used as blank control. The ratio of the absorbance at 260 nm and at 280 nm in the range of 1.8 to 2.3 is used to define good RNA quality. Total RNA of 0.5 µg mixed with 5x loading dye was subjected to 1% agarose gel electrophoresis containing 0.4 µg/ml ethidium bromide for 45 min in 1x TAE buffer at 80 V. Bands were visualized under UV light exposure in the ChemImager (Alpha Innotech, CA, USA) to check RNA integrity as well as to detect any genomic DNA contamination. Band intensity of 28S to18S ribosomal RNA subunits in a ratio of 2:1 denotes intact RNA.

Appendix VI

Positive control for IHC staining

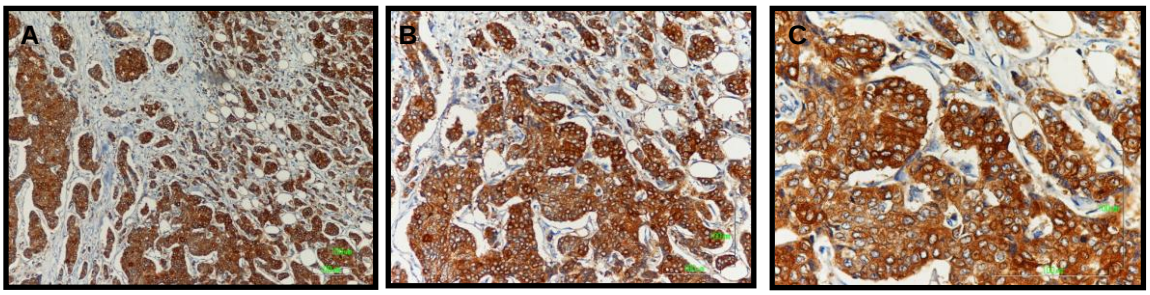


Figure 1: GNA12 expression in breast cancer tissues. GNA12 was present at high level in breast cancer tissues (A) 100x magnification (B) 200x magnification and (C) 400x magnification.

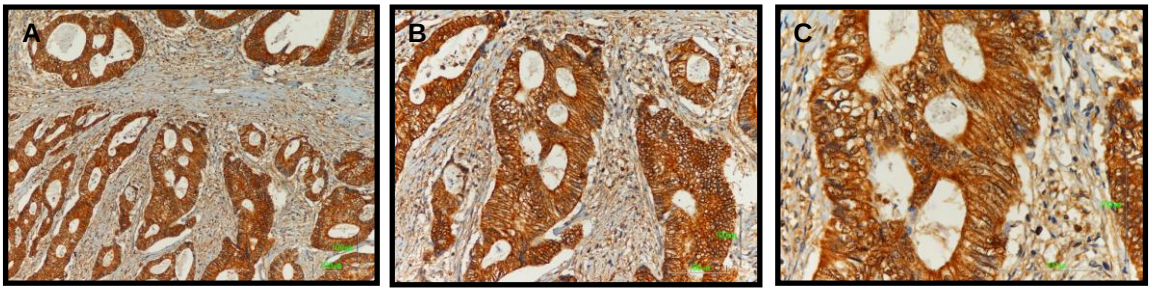


Figure 2: High level of IFITM3 detected in colorectal cancer tissues. (A) 100x magnification (B) 200x magnification and (C) 400x magnification.

Reagents for IHC

10x PBS

NaCl	80.0g
$NaH_2PO_4.H_2O$	14.4g
KCl	2.0g
KH_2PO_4	2.4g

Adjust to pH 7.4

Distilled water up to 1L

Citrate buffer

Trisodium citrate	2.94g

Adjust to pH 6.0

Distilled water up to 1L

Appendix VII

Maintenance of cell line

Oral cancer cell lines were maintained in Dulbecco's Modified Eagle's Medium/Nutrient mixture F-12 HAM's (DMEM/F-12) (Hyclone, Utah, USA) complete medium, containing 1.2 g/L sodium bicarbonate (Merck, Darmstadt, Germany), 500 ng/ml hydrocortisone (Sigma, MO, USA) and 10% Fetal Bovine Serum (FBS) (Gibco, Auckland, NZ). Normal oral cell lines were cultured in KSFM medium (Gibco, Auckland, NZ) supplemented with 25 μg/ml bovine pituitary extract, 0.4 ng/ml epidermal growth factor, and 0.09 mM CaCl2.

Packaging cells 293FT (Invitrogen, CA, USA) and GP-293 (Clontech, CA, USA) was cultured in DMEM high glucose medium (Gibco, Auckland, NZ) containing 10% FBS, 25 μl of hydrocortisone, and 50 U/ml penicillin (Gibco, Auckland, NZ). In addition, 3T3 cells were cultured in DMEM-high glucose complete medium, until 70% confluent to harvest the conditioned medium. All cells were grown in tissue culture incubator. Medium was changed twice a week.

Cells were sub-cultured when approaching 70% confluency with Trypsin-EDTA solution (Gibco, Auckland, NZ) and neutralized with DMEM/F12 complete medium (Freshney, 1994). Cell count was performed by a hemacytometer, whereby 10 μl of cell suspension was pipetted into the counting chamber. Cells were enumerated at 10x objective under an inverted microscope. The number of cells in all four 1 mm square areas was counted and concentration was determined using the formulation below:

$$cell\ concentration = \frac{Total\ cell\ count\ in\ 4mm\ square}{4} \times 10^4\ cells/ml$$

Cells were pelleted by centrifugation at 1,000 rpm for 5 min and re-suspended with complete medium before being sub-cultured with the required concentration. After sub-culturing, cells in excess were aliquoted into cryo vials (Nunc, Roskilde, Denmark) at 1 E6/ml in DMEM/F-12 complete medium with 10% DMSO (Sigma, MO, USA), and frozen gradually in slow freezing container (Nunc, Roskilde, Denmark) before storing in liquid nitrogen.

Appendix VIII

Bacteria transformation

Briefly, plasmids were added to XL1-Blue competent cells and incubated on ice for 30 min. Cells were subjected to heat shock at 42ºC for 30 sec, and further incubated on ice for another 2 min. Following this, 400 µL of LB broth (Pronadisa, Madrid, Spain) was added and cells were incubated at 37ºC, 220 rpm for 2 hours. Transformation mix of 100 µl was spread on LB agar plate containing 100 µg/ml ampicillin (Sigma, MO, USA), and incubated overnight at 37ºC for colony formation. Single colony selected from the LB agar plate was inoculated into 6 ml LB broth containing 100 µg/ml ampicillin and grown overnight as starter culture, which was then used to inoculate 150 ml of LB broth with 100 µg/ml ampicillin. Plasmids were extracted using HiSpeed plasmid MIDI kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Meanwhile, the transformed XL1-Blue cells were cryopreserved in LB broth with 15% glycerol (BDH, Poole, UK) and stored in -80ºC.

Appendix IX

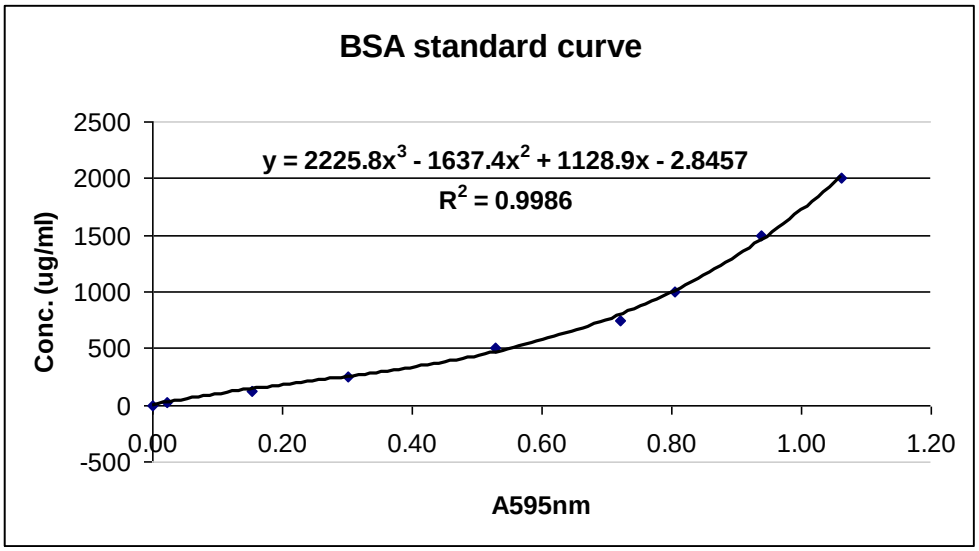
SDS-PAGE and Western Blots

(A) Protein extraction from cell lines

Oral cells were cultured in DMEM/F-12 complete medium until 70% confluent. Cells were lysed using cold RIPA lysis buffer containing 1% Halt™ 100x Protease Inhibitor Cocktail (Pierce, IL, USA), and 0.5% 200 mM Na_3VO_4 (Sigma, MO, USA). In general, 100 µl of RIPA buffer was used per 100x20 mm dish. Cells were incubated with RIPA buffer on ice with gentle rocking for 15 min. Cell lysates were homogenized and DNA was sheared by passing the lysate through a 21-gauge needle for a few times. After centrifugation at 13,000g for 15 min at 4ºC, supernatants were harvested and stored at -80ºC for further analysis.

(B) Protein quantification

Protein concentration was determined using modified Bradford assay. Briefly, 300 µl Coomassie Plus reagent (Pierce, IL, USA) was incubated with 10 µl of protein samples in a 96-well plate format for 10 min and absorbance was measured at 595 nm wavelength in a microplate reader (Thermo Fisher scientific, MA, USA). All samples were measured in triplicates and blank control was used to subtract background noise. Protein standards prepared from bovine serum albumin (BSA) with concentrations ranging between 0 to 2000µg were used to produce the standard curve for the determination of protein concentration of the samples as below:



Appendix IX, continued

(C) SDS polyacrylamide gel electrophoresis

Crude protein was mixed with 6x Laemmli buffer and denatured at 100ºC for 10 min to ensure that the proteins are in their denatured conformations. Protein samples of 50 μg were electrophoresed together with BenchMark Prestained protein ladder (Invitrogen, CA, USA) on a 4% stacking gel and separated in 12% resolving gel. Electrophoresis was carried out for 90 min at 120 V in SDS/Tris-glycine running buffer.

(D) Western blotting

Proteins were transferred onto 0.45 micron nitrocellulose membrane (Pierce, IL, USA) using the Mini Trans-Blot Electrophorectic Transfer Cell (Bio-Rad, CA, USA) in wet condition at 400 mA for 60 min. Membranes were blocked with 5% skimmed milk/TBST to prevent non-specific binding of antibodies to the membrane. Then, the following primary antibodies were used to probe the membrane: GNA12 at 1:200 dilution (sc-409, Santa Cruz, USA) and IFITM3 at 1:1000 dilution (H00010410-M01, Abnova, Taiwan). Membranes were washed thoroughly with TBST before further probing it with the respective secondary antibodies conjugated to IRDye at 1:10000 dilution (LICOR, Nebraska, USA). Protein bands were detected by infra-red detection method using the Odyssey scanner (LICOR, Nebraska, USA), and the intensity of the protein bands were measured by densitometry using the Odyssey software (LICOR, Nebraska, USA).

Blots were stripped using 0.2% NaOH for 10 min and probed with anti-actin monoclonal antibody at 1:1000 dilution (MAB1501, Chemicon, USA) to control for loading variation, and further processed as described above.

Appendix IX, continued

Reagents for SDS-PAGE and Western blotting

12% resolving gel	
Component	Volume (ml)
Water	3.3
1.5M Tris HCl (pH 8.8)	2.5
10% SDS	0.1
30% Acrylamide	4.0
10% Ammonium persulfate	0.1
TEMED	0.004

5% stacking gel	
Component	Volume (ml)
Water	3.4
1M Tris HCl (pH 6.8)	0.63
10% SDS	0.05
30% Acrylamide	0.83
10% Ammonium persulfate	0.05
TEMED	0.005

6x SDS gel loading buffer

300mM Tris-HCl, pH 6.8
600mM Dithiothreitol
12% (w/v) SDS
0.6% bromophenol blue
60% (v/v) glycerol

10x TBS

NaCl	80g
KCl	2g
Tris base	30g

Adjust to pH 8.0
Distilled water up to 1L

RIPA lysis buffer

50mM Tris-HCl pH7.4
1% NP-40
150mM NaCl
0.25% Na-deoxycholate
1mM EDTA

Transfer buffer

24mM Tris base
192mM glycine
20% methanol

1x Tris-glycine running buffer

25mM Tris-base
250mM Glycine, pH8.3
0.1% (w/v) SDS

6x Lameali buffer

SDS	1.2g
Bromophenol blue	1.5mg
Glycerol	4.7ml
0.5M Tris pH6.8	1.2ml
Sterile water	2.1ml
DTT	0.93g

Appendix X

Selection of oral cancer cell line for in-vitro functional studies

(A) GNA12

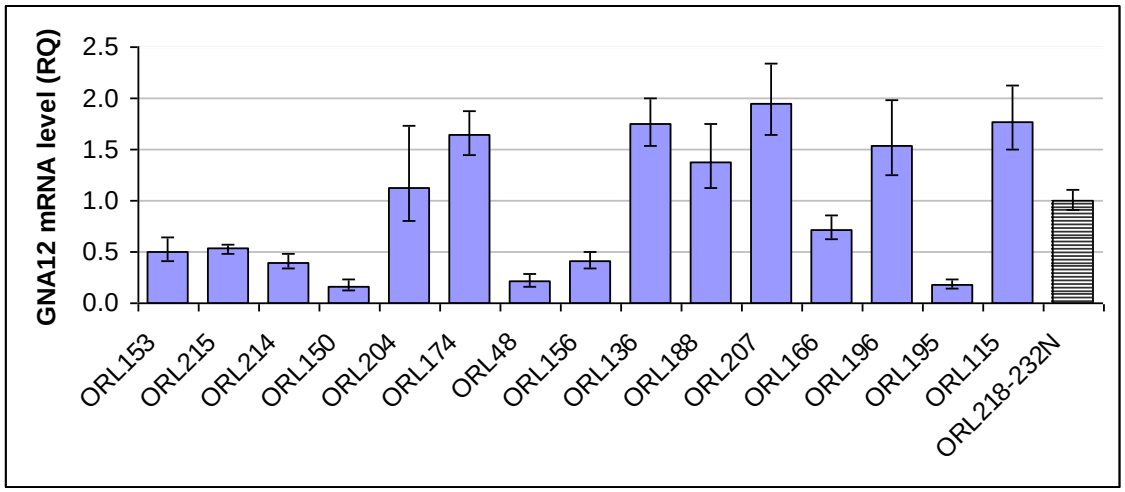


Figure 1: GNA12 mRNA expression in oral cancer cell lines. The mRNA level of GNA12 was normalized against GAPDH housekeeping gene. GNA12 expression in 15 oral cancer cell lines was calibrated against 2 non-malignant primary keratinocytes (ORL218 and ORL232N). A RQ value of ≥ 2 indicates over-expression. All cultured cell lines displayed basal expression of GNA12. Cell line ORL150 was among those with low level of GNA12, hence was selected for exogenous expression studies.

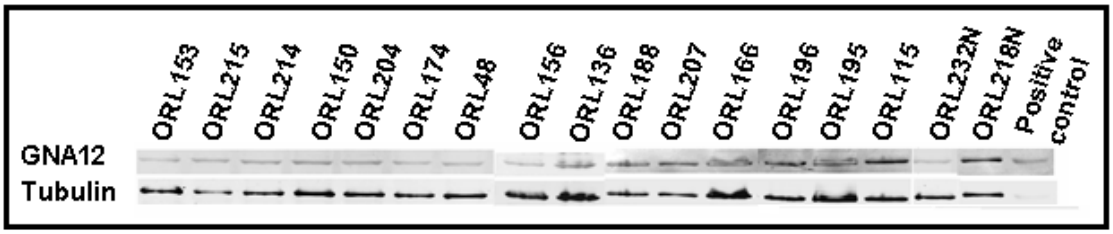


Figure 2: GNA12 protein expression in oral cancer cell lines. Basal expression of GNA12 was found in all cell lines, including the non-malignant primary keratinocytes (ORL218N and ORL232N). Protein lysate harvested from 293T cells transfected with GNA12 was used as positive control.

Appendix X, continued

(B) IFITM3

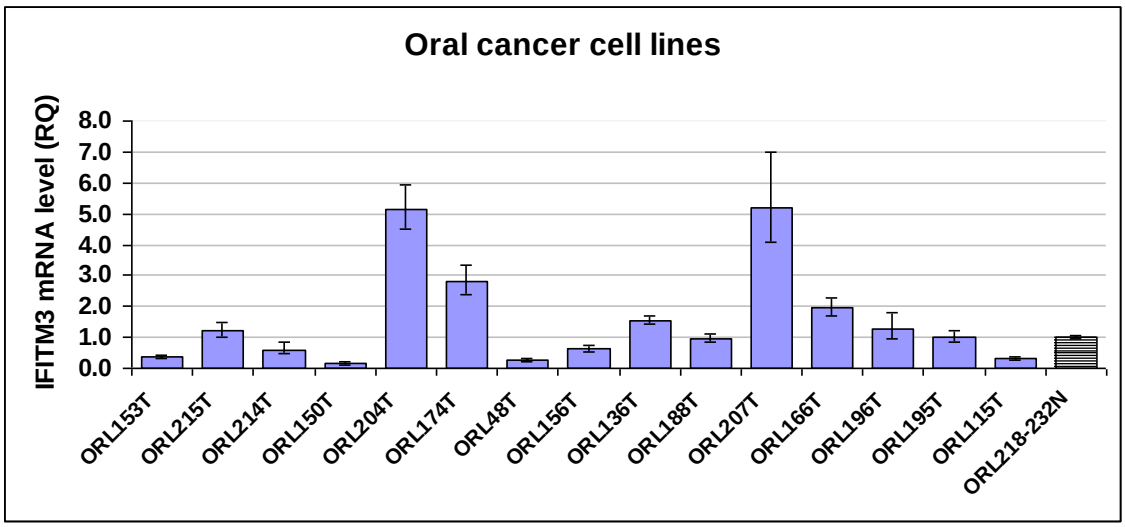


Figure 3: IFITM3 mRNA expression in oral cancer cell lines. The mRNA level of IFITM3 was normalized against GAPDH housekeeping gene. IFITM3 expression in 15 oral cancer cell lines was calibrated against 2 non-malignant primary keratinocytes (ORL218 and ORL232N). Over-expression was determined at RQ ≥ 2. ORL207 cell line has the highest expression of IFITM3, whilst ORL188 demonstrated low level of IFITM3 expression.

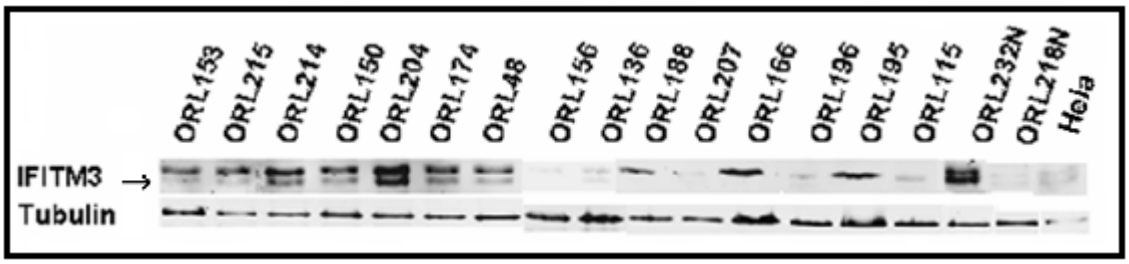
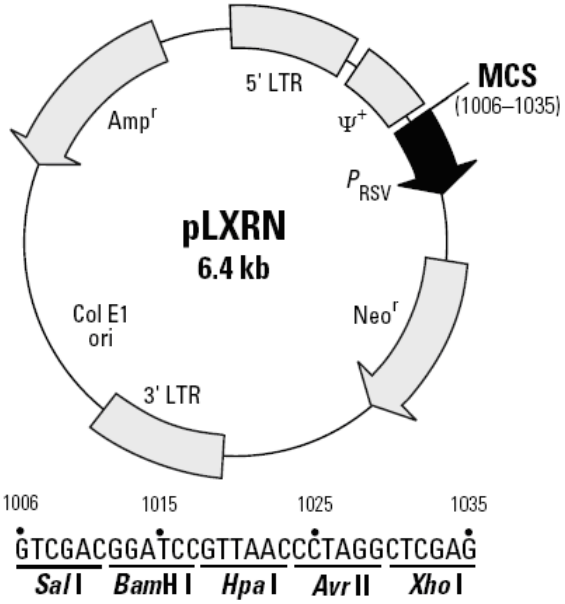


Figure 4: IFITM3 protein expression in oral cancer cell lines. Expression of IFITM3 is indicated by the presence of the lower band. Majority of the oral cancer cell lines showed high levels of IFITM3 compared to the ORL218N. Meanwhile, ORL232N showed high basal level of IFITM3. Low level of IFITM3 protein was also detected in ORL188. Protein lysates from Hela cell line was used as positive control.

Appendix XI

pLXRN retroviral vector information (Clontech)



Location of features:

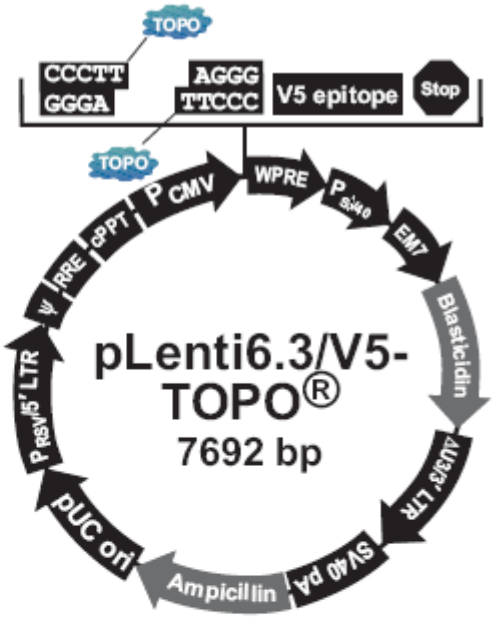
- 5’ MoMuSV LTR: 1-589
- Ψ^+ (packaging signal): 659-989
- Multiple cloning site (MCS): 1006-1035
- Rous sarcoma virus (RSV) promoter (P_{RSV}): 1039-1350
- Neomycin resistance gene (Neo^r):
  start codon: 1704-1706; stop codon: 2496-2498
- 3’ MoMuSV LTR: 3290-3883
- Col E1 origin of replication: 4419
- Ampicillin resistance gene (β-lactamase): start codon: 5181-5179

Propagation in *E. coli*

- Suitable host strains: DH5α, HB101 and other general purpose strains
- Selectable marker: plasmid confers resistance to ampicillin (100μg/ml) to *E. coli* host
- *E.coli* replication origin: Col E1
- Copy number: low

Appendix XII

pLenti6.3 lentiviral vector information (Invitrogen)



Comments for pLenti6.3/V5-TOPO
7692 nucleotides

RSV/5′ LTR hybrid promoter: bases 1-410
RSV promoter: bases 1-229
HIV-1 5′ LTR: bases 230-410
HIV-1 psi (ψ) packaging signal: bases 521-565
HIV-1 Rev response element (RRE): bases 1075-1308
cPPT: bases 1800-1922
CMV promoter: bases 1934-2518
TOPO® cloning site: bases 2557-2566
V5 epitope: bases 2629-2670
WPRE: bases 2689-3286
SV40 promoter: bases 3297-3605
EM7 promoter: bases 3660-3726
Blasticidin resistance gene: bases 3727-4125
ΔU3/3′ LTR: bases 4211-4445
ΔU3: bases 4211-4264
3′ LTR: bases 4265-4445
SV40 polyadenylation signal: bases 4517-4648
bla promoter: bases 5507-5605
Ampicillin (*bla*) resistance gene: bases 5606-6466
pUC origin: bases 6611-7284

Appendix XIII

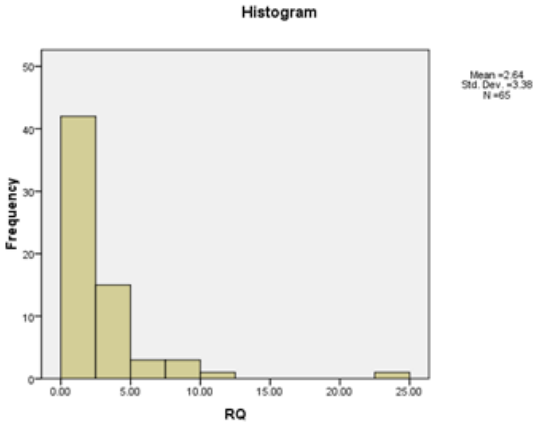
Normality test on sample distribution

Test of normality for GNA12 QPCR analysis

Tests of Normality

	Kolmogorov-Smimov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
RQ	.269	65	.000	.560	65	.000

a. Lilliefors Significance Correction

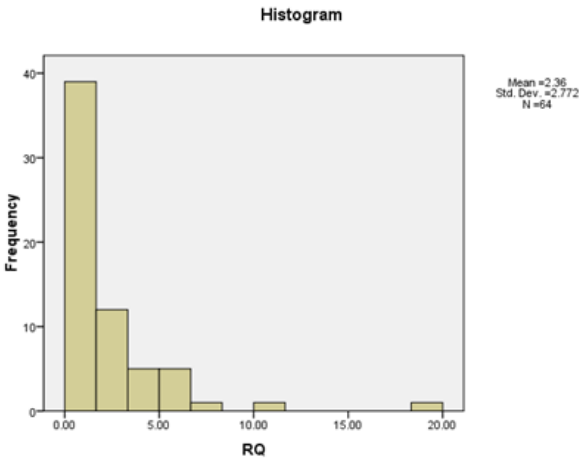


Test of normality for IFITM3 QPCR analysis

Tests of Normality

	Kolmogorov-Smimov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
RQ	.247	64	.000	.595	64	.000

a. Lilliefors Significance Correction



Appendix XIII, continued

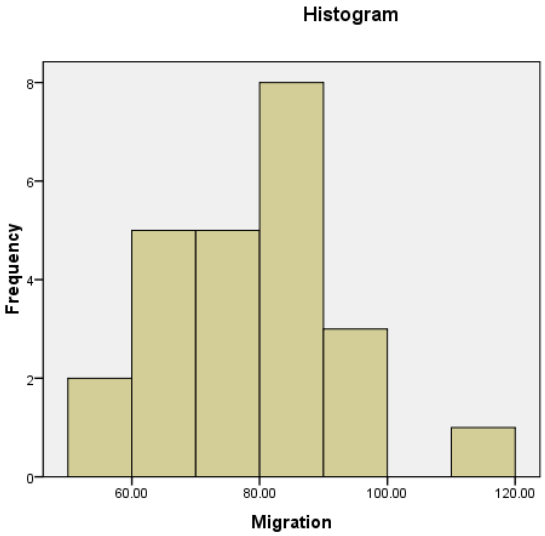
Test of normality for monolayer scratch assay

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Migration	.098	24	.200*	.967	24	.590

a. Lilliefors Significance Correction

*. This is a lower bound of the true significance.



Test of normality for Transwell invasion assay

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Invasion	.324	6	.048	.844	6	.141

a. Lilliefors Significance Correction