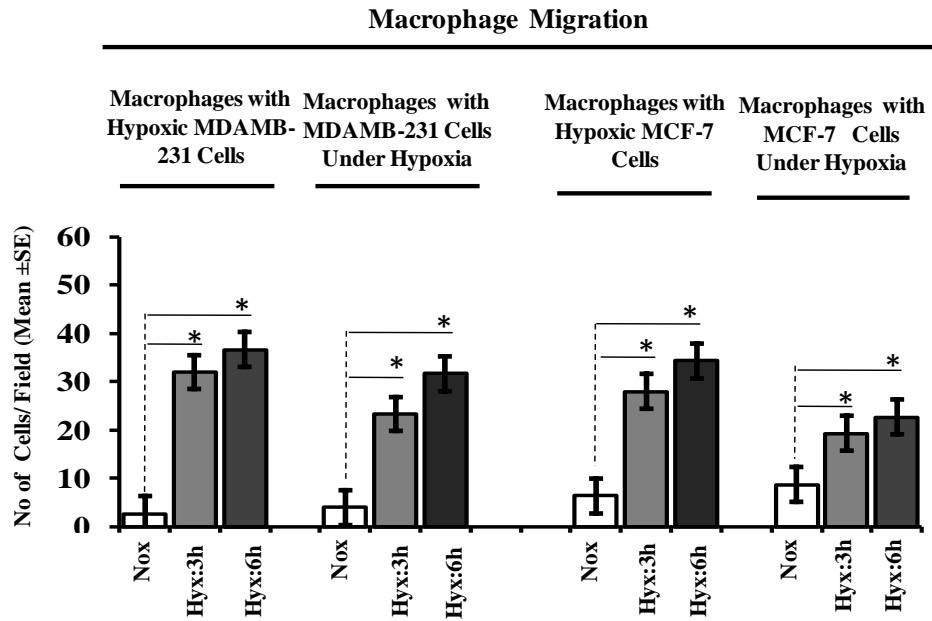


Macrophages are recruited to hypoxic tumor areas and acquire a Pro-Angiogenic M2-Polarized phenotype via hypoxic cancer cell derived cytokines Oncostatin M and Eotaxin

Supplemental Information



Suppl.1: Comparison Chemotactic Data for Macrophage Co-Culture Hypoxic Cancer Cells Vs Macrophage/Cancer Cells Co-Culture Subject to Hypoxia

Suppl.2: RayBio Human Cytokine Antibody Array (5) MAP

	1	2	3	4	5	6	7	8
A	GRO- α	IL-10	MIP-1 β	Angiogenin	Eotaxin-3	IGFBP-2	NT-3	Pos
B	GRO	IL-8	MIG	IGF-1	Eotaxin-2	IGFBP-1	NAP-2	Pos
C	GM-CSF	IL-7	MDC	EGF	Eotaxin	HGF	MIP-3 α	TIMP-2
D	GCSF	IL-6	MCSF	TNF- β	Ck β 8-1	GDNF	MIF	TIMP-1
E	ENA-78	IL-5	MCP-3	TNF- α	BLC	GCP-2	MCP-4	TGF- β 3
F	Neg	IL-4	MCP-2	TGF- β 1	BDNF	Fractalkine	LIGHT	TGF- β 2
G	Neg	IL-3	MCP-1	TARC	Leptin	Flt-3 Ligand	LIF	PIGF
H	Pos	IL-2	IFN- γ	SDF-1	PDGF-BB	FGF-9	IP-10	PARC
I	Pos	IL-1 β	IL-15	SCF	VEGF	FGF-7	IL-16	Osteoprotegerin
J	Pos	IL-1 α	IL-13	RANTES	Thrombopoietin	FGF-6	IGFBP-4	Osteopontin
K	Pos	I-309	IL-12p40p70	MIP-1 δ	OncostatinM	FGF-4	IGFBP-3	NT-4

No.	MCF-7	MDAMB-231
1.	OncostatinM	OncostatinM
2.	-	RANTES
3.	FGF-6	-
4.	VEGF	VEGF
5.	-	IL-3
6.	-	MCP-1
7.	-	TAR-C
8.	Eotaxin	Eotaxin
9.	TNF- β	TNF- β
10.	Eotaxin-2	-
11.	-	MIP-1 β
12.	IL-10	-
13.	-	Leptin

Suppl. 3: List of Human Cytokines Upregulated in Hypoxic Tumour Cell Conditioned Media in Comparison to Normoxic Tumour Cell Conditioned Media

Correlation between

HIF-1 α Vs Oncostatin M

Total no. of field Evaluated: 74

Level of HIF 1- α	High:	Low /UD:		
	41	33		
Level of Oncostatin M	High:	Low/UD:	High:	Low/UD:
	41	0	2	31

Chi square test with (yates corrected) Significant (p < .001) association between HIF-1 α and Oncostatin M

Correlation between

Oncostatin M Vs CD206

Total no. of field Evaluated: 60

Level of Oncostatin M	High:	Low /UD:		
	33	27		
Level of CD206	High:	Low/UD:	High:	Low/UD:
	33	0	1	26

Chi square test with (yates corrected) Significant (p < .001) association between Oncostatin M and CD206

Correlation between

HIF-1 α Vs Eotaxin

Total no. of field Evaluated: 65

Level of HIF 1- α	High:	Low /UD:		
	36	29		
Level of Eotaxin	High:	Low/UD:	High:	Low/UD:
	36	0	2	27

Chi square test with (yates corrected) Significant (p < .001) association between HIF-1 α and Eotaxin

Correlation between

Eotaxin Vs CD206

Total no. of field Evaluated: 54

Level of Eotaxin	High:	Low /UD:		
	31	23		
Level of CD206	High:	Low/UD:	High:	Low/UD:
	29	2	1	22

Chi square test with (yates corrected) Significant (p < .001) association between Eotaxin and CD206

Numbers represent fields showing presence of indicated antigen evaluated; UD =Undetectable level.

Suppl.4: Collective summary of Immunohistochemical Observations of all (14) the Human Breast Cancer Specimen

Patient wise correlation between

HIF-1 α Vs Oncostatin M

Patient No. 3	Total no. of field Evaluated: 11				
	Level of HIF 1- α	High:	Low /UD:		
		7	4		
Patient No. 25	Total no. of field Evaluated:4				
	Level of Oncostatin M	High:	Low/UD:	High:	Low/UD:
		7	0	0	4
Patient No. 28	Total no. of field Evaluated:5				
	Level of HIF 1- α	High:	Low /UD:		
		4	0		
Patient No. 41	Total no. of field Evaluated:5				
	Level of Oncostatin M	High:	Low/UD:	High:	Low/UD:
		2	0	0	3
Patient No. 42	Total no. of field Evaluated:5				
	Level of HIF 1- α	High:	Low /UD:		
		2	3		
Patient No. 3	Total no. of field Evaluated:4				
	Level of Oncostatin M	High:	Low/UD:	High:	Low/UD:
		2	0	0	2

Patient wise correlation between

Oncostatin M Vs CD206

Patient No. 3	Total no. of field Evaluated: 11				
	Level of Oncostatin M	High:	Low /UD:		
		7	4		
Patient No. 25	Total no. of field Evaluated:3				
	Level of CD206	High:	Low/UD:	High:	Low/UD:
		7	0	0	4
Patient No. 28	Total no. of field Evaluated:5				
	Level of Oncostatin M	High:	Low /UD:		
		5	0		
Patient No. 41	Total no. of field Evaluated:5				
	Level of CD206	High:	Low/UD:	High:	Low/UD:
		5	0	0	0
Patient No. 41	Total no. of field Evaluated:5				
	Level of Oncostatin M	High:	Low /UD:		
		3	2		
Patient No. 42	Total no. of field Evaluated:5				
	Level of CD206	High:	Low/UD:	High:	Low/UD:
		3	0	0	2
Patient No. 3	Total no. of field Evaluated:11				
	Level of Oncostatin M	High:	Low /UD:		
		7	4		
Patient No. 25	Total no. of field Evaluated:3				
	Level of Oncostatin M	High:	Low /UD:		
		2	1		
Patient No. 28	Total no. of field Evaluated:5				
	Level of Oncostatin M	High:	Low /UD:		
		2	0		
Patient No. 41	Total no. of field Evaluated:5				
	Level of Oncostatin M	High:	Low /UD:		
		2	3		
Patient No. 42	Total no. of field Evaluated:5				
	Level of Oncostatin M	High:	Low /UD:		
		2	3		

Total no. of field Evaluated:5

Patient No.43	Level of HIF 1- α	High:		Low /UD:	
		2		3	
	Level of Oncostatin M	High:	Low/UD:	High:	Low/UD:
		2	0	0	3
	Total no. of field Evaluated:10				

Total no. of field Evaluated:10

Patient No. 46	Level of HIF 1- α	High:		Low /UD:	
		7		3	
	Level of Oncostatin M	High:	Low/UD:	High:	Low/UD:
		7	0	0	3
	Total no. of field Evaluated:2				

Total no. of field Evaluated:2

Patient No. 47	Level of HIF 1- α	High:		Low /UD:	
		1		1	
	Level of Oncostatin M	High:	Low/UD:	High:	Low/UD:
		1	0	0	1
	Total no. of field Evaluated: 10				

Total no. of field Evaluated:10

Patient No. 51	Level of HIF 1- α	High:		Low /UD:	
		6		4	
	Level of Oncostatin M	High:	Low/UD:	High:	Low/UD:
		6	0	0	4
	Total no. of field Evaluated:2				

Total no. of field Evaluated:2

Patient No. 52	Level of HIF 1- α	High:		Low /UD:	
		1		1	
	Level of Oncostatin M	High:	Low/UD:	High:	Low/UD:
		1	0	0	1
	Total no. of field Evaluated:4				

Total no. of field Evaluated:4

Patient No. 56	Level of HIF 1- α	High:		Low /UD:	
		3		1	

Total no. of field Evaluated:4

Patient No. 43	Level of Oncostatin M	High:		Low /UD:	
		1		3	
	Level of CD206	High:	Low/UD:	High:	Low/UD:
		1	0	0	3
	Total no. of field Evaluated:3				

Total no. of field Evaluated:3

Patient No. 46	Level of Oncostatin M	High:		Low /UD:	
		1		2	
	Level of CD206	High:	Low/UD:	High:	Low/UD:
		1	0	1	1
	Total no. of field Evaluated:3				

Total no. of field Evaluated:3

Patient No. 47	Level of Oncostatin M	High:		Low /UD:	
		1		2	
	Level of CD206	High:	Low/UD:	High:	Low/UD:
		1	0	0	2
	Total no. of field Evaluated: 3				

Total no. of field Evaluated:3

Patient No. 51	Level of Oncostatin M	High:		Low /UD:	
		2		1	
	Level of CD206	High:	Low/UD:	High:	Low/UD:
		2	0	0	1
	Total no. of field Evaluated:2				

Total no. of field Evaluated:2

Patient No. 52	Level of Oncostatin M	High:		Low /UD:	
		1		1	
	Level of CD206	High:	Low/UD:	High:	Low/UD:
		1	0	0	1
	Total no. of field Evaluated:5				

Total no. of field Evaluated:5

Patient No. 56	Level of Oncostatin M	High:		Low /UD:	
		2		3	

Patient No. 57	Level of Oncostatin M	High: 3	Low/UD: 0	High: 0	Low/UD: 1
	Total no. of field Evaluated:6				
	Level of HIF 1- α	High: 3	Low /UD: 3		
Patient No. 59	Level of Oncostatin M	High: 3	Low/UD: 0	High: 1	Low/UD: 2
	Total no. of field Evaluated:3				
	Level of HIF 1- α	High: 1	Low /UD: 2		
Patient No. 60	Level of Oncostatin M	High: 1	Low/UD: 0	High: 0	Low/UD: 2
	Total no. of field Evaluated:3				
	Level of HIF 1- α	High: 0	Low /UD: 3		
Patient No. 60	Level of Oncostatin M	High: 0	Low/UD: 0	High: 0	Low/UD: 3

Patient No. 57	Level of CD206	High: 2	Low/UD: 0	High: 0	Low/UD: 3
	Total no. of field Evaluated:6				
	Level of Oncostatin M	High: 2	Low /UD: 4		
Patient No. 59	Level of CD206	High: 2	Low/UD: 0	High: 0	Low/UD: 4
	Total no. of field Evaluated:3				
	Level of Oncostatin M	High: 2	Low /UD: 1		
Patient No. 60	Level of CD206	High: 2	Low/UD: 0	High: 0	Low/UD: 1
	Total no. of field Evaluated:2				
	Level of Oncostatin M	High: 0	Low /UD: 2		
Patient No. 60	Level of CD206	High: 0	Low/UD: 0	High: 0	Low/UD: 2

Patient wise correlation between HIF-1 α Vs Eotaxin

Patient wise correlation between Eotaxin Vs CD206

Patient No. 3	Total no. of field Evaluated: 6				
	Level of HIF 1- α	High: 4	Low /UD: 2		
	Level of Eotaxin	High: 4	Low/UD: 0	High: 0	Low/UD: 2
Patient No. 25	Total no. of field Evaluated:5				
	Level of HIF 1- α	High: 3	Low /UD: 2		

Patient No. 3	Total no. of field Evaluated: 3				
	Level of Eotaxin	High: 2	Low /UD: 1		
	Level of CD206	High: 2	Low/UD: 0	High: 0	Low/UD: 1
Patient No. 25	Total no. of field Evaluated:4				
	Level of Eotaxin	High: 2	Low /UD: 2		

Patient No. 28	Level of Eotaxin	High: 3	Low/UD: 0	High: 0	Low/UD: 2
	Total no. of field Evaluated:5				
Patient No. 41	Level of HIF 1- α	High: 2	Low /UD: 3		
	Level of Eotaxin	High: 2	Low/UD: 0	High: 0	Low/UD: 3
Patient No. 42	Level of HIF 1- α	High: 3	Low /UD: 3		
	Level of Eotaxin	High: 3	Low/UD: 0	High: 0	Low/UD: 3
Patient No. 43	Level of HIF 1- α	High: 3	Low /UD: 3		
	Level of Eotaxin	High: 3	Low/UD: 0	High: 1	Low/UD: 2
Patient No. 46	Level of HIF 1- α	High: 4	Low /UD: 0		
	Level of Eotaxin	High: 4	Low/UD: 0	High: 0	Low/UD: 0

Patient No. 28	Level of CD206	High: 2	Low/UD: 0	High: 0	Low/UD: 2
	Total no. of field Evaluated:5				
Patient No. 41	Level of Eotaxin	High: 3	Low /UD: 2		
	Level of CD206	High: 3	Low/UD: 0	High: 0	Low/UD: 2
Patient No. 42	Level of Eotaxin	High: 2	Low /UD: 1		
	Level of CD206	High: 2	Low/UD: 0	High: 0	Low/UD: 2
Patient No. 43	Level of Eotaxin	High: 2	Low /UD: 2		
	Level of CD206	High: 1	Low/UD: 1	High: 0	Low/UD: 1
Patient No. 46	Level of Eotaxin	High: 2	Low /UD: 1		
	Level of CD206	High: 2	Low/UD: 0	High: 0	Low/UD: 2

Patient No. 51

Level of HIF 1- α	High:	Low /UD:	
	2	2	
Level of Eotaxin	High:	Low/UD:	High: Low/UD:
	2	0	1 1
Total no. of field Evaluated: 5			

Level of HIF 1- α	High:	Low /UD:	
	3	2	
Level of Eotaxin	High:	Low/UD:	High: Low/UD:
	3	0	0 2
Total no. of field Evaluated:4			

Patient No. 52

Level of HIF 1- α	High:	Low /UD:	
	1	3	
Level of Eotaxin	High:	Low/UD:	High: Low/UD:
	1	0	0 3
Total no. of field Evaluated:4			

Patient No. 56

Level of HIF 1- α	High:	Low /UD:	
	3	1	
Level of Eotaxin	High:	Low/UD:	High: Low/UD:
	3	0	0 1
Total no. of field Evaluated:5			

Patient No. 57

Level of HIF 1- α	High:	Low /UD:	
	2	3	
Level of Eotaxin	High:	Low/UD:	High: Low/UD:
	2	0	0 3
Total no. of field Evaluated:5			

Patient No. 59

Level of HIF 1- α	High:	Low /UD:	
	2	3	
Level of Eotaxin	High:	Low/UD:	High: Low/UD:

Patient No. 51

Level of Eotaxin	High:	Low /UD:	
	2	1	
Level of CD206	High:	Low/UD:	High: Low/UD:
	2	0	0 1
Total no. of field Evaluated: 5			

Level of Eotaxin	High:	Low /UD:	
	3	2	
Level of CD206	High:	Low/UD:	High: Low/UD:
	3	0	0 2
Total no. of field Evaluated:5			

Patient No. 52

Level of Eotaxin	High:	Low /UD:	
	2	3	
Level of CD206	High:	Low/UD:	High: Low/UD:
	2	0	0 3
Total no. of field Evaluated:3			

Patient No. 56

Level of Eotaxin	High:	Low /UD:	
	2	1	
Level of CD206	High:	Low/UD:	High: Low/UD:
	2	0	0 1
Total no. of field Evaluated:4			

Patient No. 57

Level of Eotaxin	High:	Low /UD:	
	3	1	
Level of CD206	High:	Low/UD:	High: Low/UD:
	2	1	0 1
Total no. of field Evaluated:4			

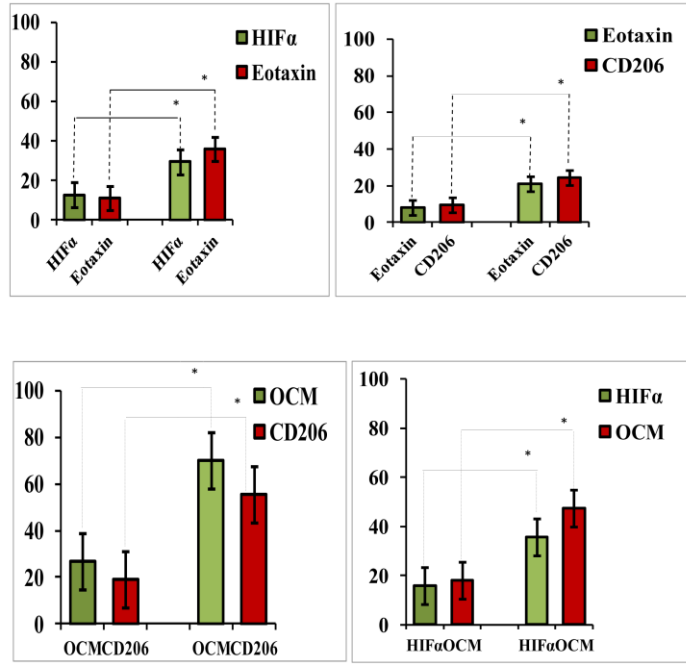
Patient No. 59

Level of Eotaxin	High:	Low /UD:	
	2	2	
Level of CD206	High:	Low/UD:	High: Low/UD:

Patient No. 60		2	0	0	3	Patient No. 60		2	0	0	2	
		Total no. of field Evaluated:3						Total no. of field Evaluated:4				
	Level of HIF 1- α	High:		Low /UD:				Level of Eotaxin	High:		Low /UD:	
		2			1				2			2
	Level of Eotaxin	High:	Low/UD:	High:	Low/UD:		Level of CD206	High:	Low/UD:	High:	Low/UD:	
		2	0	0	1			2	0	0	2	

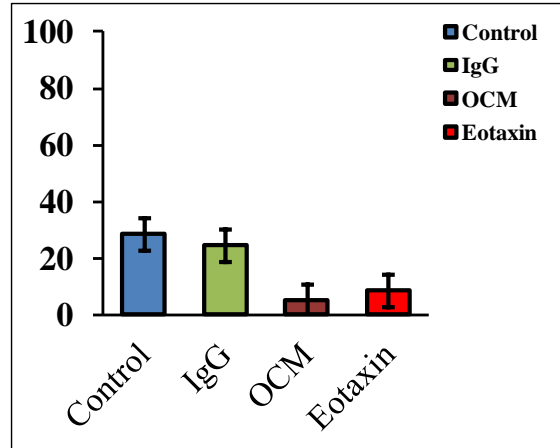
Numbers represent fields showing presence of indicated antigen evaluated; UD =Undetectable level.

Suppl. 5: Summary of Immunohistochemical Observations of Individual Human Breast Cancer Specimen



Each bar represents the mean intensity of five different fields.

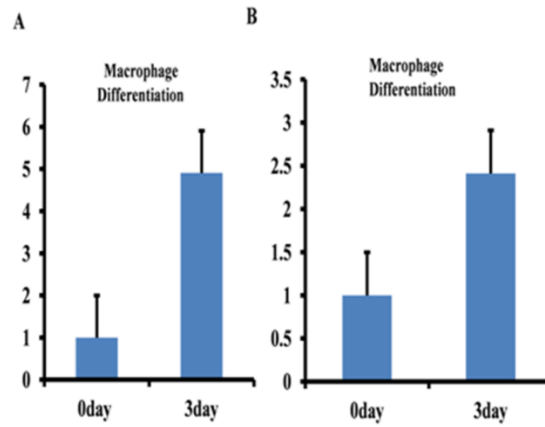
Suppl.6: Quantification of Oncostatin M/Eotaxin and CD206 Expression (Florescence intensity) in Hypoxic (HIF-1α Positive) area of Human Breast Cancer Specimen



Each bar represents the mean intensity of five different fields.

Suppl.7: Quantification of CD31 Expression (Florescence intensity) in 4T1/BALB/c Tumour Tissue

(A) Mc-I-1 (B) CD16 Expression



Suppl. 8: Real-Time Quantitative PCR of Macrophage Specific Markers in THP-1 Derived Macrophages