

Lung resident mesenchymal cells isolated from patients with the Bronchiolitis Obliterans Syndrome display a deregulated epigenetic profile.

Serena Vella^{1,2,*}, Pier Giulio Conaldi^{1,3}, Emanuela Cova⁴, Federica Meloni⁴, Rosa Liotta⁵, Salvatore Cuzzocrea⁶, Lavinia Martino⁷, Alessandro Bertani⁷, Angelo Luca⁸, Patrizio Vitulo⁷

¹Department of Laboratory Medicine and Advanced Biotechnologies, IRCCS-ISMETT (Istituto Mediterraneo per i Trapianti e Terapie ad Alta Specializzazione), Palermo, Italy.

²Current address: Anemocyte S.r.l., Gerenzano, Italy.

³ Fondazione Ri.MED, Palermo, Italy.

⁴Department of Respiratory Diseases, IRCCS San Matteo Foundation and University of Pavia, Pavia, Italy.

⁵Department of Diagnostic and Therapeutic Services, Pathology Service, IRCCS-ISMETT (Istituto Mediterraneo per i Trapianti e Terapie ad Alta Specializzazione), Palermo, Italy.

⁶Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy.

⁷Department for the Treatment and Study of Cardiothoracic Diseases and Cardiothoracic Transplantation, IRCCS-ISMETT (Istituto Mediterraneo per i Trapianti e Terapie ad Alta Specializzazione), Palermo, Italy.

⁸Department of Diagnostic and Therapeutic Services, Radiology Service, IRCCS-ISMETT (Istituto Mediterraneo per i Trapianti e Terapie ad Alta Specializzazione), Palermo, Italy.

*** Corresponding author:**

Serena Vella

Email: vellaserena@gmail.com

Supplementary Table S1: Clinical characteristics of patients.

MSC from BALf	Sample	Age	Gender	Diagnosis	Type of LTx	BOS
Stable LTRs	4069	44	M	NSIP	Single	No
	4175	65	M	UIP	Single	No
	4205	44	F	NSIP	Single	No
	3887	57	M	CPFE	Bilateral	No
	3911	71	M	E	Bilateral	No
	Median (range)		56.2 (12.19)			
BOS	4352	66	F	NSIP	Single	Yes (BOS0p)
	3780	38	F	NSIP	Single	Yes (BOS0p)
	3649	58	M	RF	Single	Yes (BOS1)
	4000	38	M	CF	Bilateral	Yes (BOS1)
	3995	38	M	PPH	Bilateral	Yes (BOS3)
	Median (range)		47.6 (13.45)			

FFPE lung biopsy	Sample	Age	Gender	Diagnosis	Type of LTx	BOS
Stable LTRs	40959	59	M	IPF	Bilateral	No
	30898	59	M	IPF	Single	No
	25174	27	M	LCH	Bilateral	No
	33526	43	M	IPF	Bilateral	No
	Average (SD)		47 (15.32)			
BOS	34368	52	M	CF	Bilateral	Yes (C1)
	30584	66	M	IPF	Bilateral	Yes (A0BOC1)
	36465	46	M	IPF	Bilateral	Yes (A3B1C1)
	32011	47	F	IPF	Bilateral	Yes (A1B2C1)
	Median (range)		52.75 (9.21)			

NSIP: Non-specific interstitial pneumonia
UIP: Usual interstitial pneumonia
CPFE: Combined pulmonary fibrosis and emphysema
E: Emphysema
RF: Radiation Fibrosis
PPH: Primary pulmonary hypertension
CF: Cystic fibrosis
LCH: Langerhans cell histiocytosis

Supplementary Table S2. Genes over-expressed in MSC from BOS 0p patients vs Control Group (fold change ≥ 1.5).

Gene	Fold Change	p-value
RPS6KA5	2.9233	0.023325
HDAC2	1.7719	0.000605
SETDB2	1.7554	0.541659
DZIP3	1.7383	0.016134
PRMT6	1.723	0.079819
RNF2	1.7164	0.012637
MECP2	1.7077	0.841718
HDAC1	1.7061	0.087215
HDAC3	1.5958	0.001826
NCOA1	1.5722	0.07958
HDAC5	1.5676	0.107408
RPS6KA3	1.5614	0.007886
MBD2	1.5332	0.050273
CIITA	1.5122	0.652307
UBE2A	1.5078	0.027294

Supplementary Table S3. Genes down-regulated in MSC from BOS 0p patients vs Control Group (fold change ≥ 0.5).

Gene	Fold Change	p-value
ESCO2	0.4227	0.131933
AURKB	0.4264	0.121206

Supplementary Table S4. Genes over-expressed in MSC from BOS patients vs Control Group (fold change > 1.5).

Gene	Fold Change	p-value
RPS6KA5	3.281	0.017646
CIITA	2.7344	0.001783
DZIP3	2.2063	0.000488
SETDB2	2.0777	0.161377
MECP2	2.068	0.295311
HDAC2	2.0453	0.000845
PRMT8	1.9715	0.031952
HDAC1	1.9667	0.012195
UBE2A	1.8869	0.003514
RNF2	1.8239	0.012785
PRMT3	1.7812	0.000519
UBE2B	1.727	0.011008
USP16	1.7247	0.007111
NEK6	1.6612	0.128402
EZH2	1.6297	0.306982
MYSM1	1.6281	0.002822
HDAC3	1.6235	0.000007
DNMT3B	1.5827	0.020132
DNMT1	1.5812	0.403073
KAT6A	1.5705	0.060173
HDAC8	1.5428	0.008882
KAT6B	1.5278	0.027449
NCOA6	1.5105	0.006834

Supplementary Table S5. GO molecular function of genes over-expressed in MSC from BOS patients vs Control Group (fold change > 1.5).

GO molecular function	Genes
DNA-methyltransferase activity (GO:0009008)	DNMT3B, DNMT1
DNA (cytosine-5-)-methyltransferase activity, acting on CpG substrates (GO:0051718)	DNMT3B, DNMT1
DNA (cytosine-5-)-methyltransferase activity (GO:0003886)	DNMT3B, DNMT1
unmethylated CpG binding (GO:0045322)	HDAC1, HDAC2, HDAC3
NF-kappaB binding (GO:0051059)	DNMT3B, DNMT1, MBD2
structure-specific DNA binding (GO:0043566)	MBD2, HDAC1, HDAC2, EZH2
protein methyltransferase activity (GO:0008276)	HDAC1, HDAC2, HDAC3, HDAC8
protein deacetylase activity (GO:0033558)	HDAC1, HDAC2, HDAC3, HDAC8
N-methyltransferase activity (GO:0008170)	SETDB2, PRMT3, EZH2, PRMT8
NAD-dependent protein deacetylase activity (GO:0034979)	HDAC1, HDAC2, HDAC3, HDAC8
NAD-dependent histone deacetylase activity (H3-K14 specific) (GO:0032041)	HDAC1, HDAC2, HDAC3, HDAC8
NAD-dependent histone deacetylase activity (GO:0017136)	HDAC1, HDAC2, HDAC3, HDAC8
hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds, in linear amides (GO:0016811)	HDAC1, HDAC2, HDAC3, HDAC8
hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds (GO:0016810)	HDAC1, HDAC2, HDAC3, HDAC8
histone methyltransferase activity (GO:0042054)	SETDB2, PRMT3, EZH2, PRMT8
histone deacetylase activity (H3-K14 specific) (GO:0031078)	HDAC1, HDAC2, HDAC3, HDAC8
histone deacetylase activity (GO:0004407)	HDAC1, HDAC2, HDAC3, HDAC8
deacetylase activity (GO:0019213)	HDAC1, HDAC2, HDAC3, HDAC8
chromatin DNA binding (GO:0031490)	HDAC1, HDAC2, MBD2, EZH2

transcription coactivator activity (GO:0003713)	NCOA6, USP16, CIITA, KAT6A, MYSM1
histone deacetylase binding (GO:0042826)	DNMT3B, MBD2, HDAC3, HDAC1, DNMT1
transferase activity, transferring one-carbon groups (GO:0016741)	DNMT3B, DNMT1, SETDB2, PRMT3, EZH2, PRMT8
S-adenosylmethionine-dependent methyltransferase activity (GO:0008757)	DNMT3B, DNMT1, SETDB2, PRMT3, EZH2, PRMT8
methyltransferase activity (GO:0008168)	DNMT3B, DNMT1, SETDB2, PRMT3, EZH2, PRMT8
transcription factor binding (GO:0008134)	MBD2, NCOA6, KAT6B, HDAC3, HDAC1, CIITA, HDAC8, KAT6A, HDAC2
transcription factor activity, transcription factor binding (GO:0000989)	DNMT3B, MBD2, NCOA6, USP16, HDAC3, HDAC1, CIITA, KAT6A, MYSM1
transcription factor activity, protein binding (GO:0000988)	DNMT3B, MBD2, NCOA6, USP16, HDAC3, HDAC1, CIITA, KAT6A, MYSM1
transcription cofactor activity (GO:0003712)	DNMT3B, MBD2, NCOA6, USP16, HDAC3, HDAC1, CIITA, KAT6A, MYSM1
chromatin binding (GO:0003682)	DNMT3B, MBD2, NCOA6, HDAC3, HDAC1, DNMT1, RNF2, HDAC2, EZH2
enzyme binding (GO:0019899)	UBE2A, DNMT3B, MBD2, DZIP3, NCOA6, NEK6, UBE2B, HDAC3, HDAC1, DNMT1, HDAC2
macromolecular complex binding (GO:0044877)	DNMT3B, MBD2, NCOA6, KAT6B, HDAC3, HDAC1, CIITA, RNF2, HDAC2, MYSM1, EZH2
transferase activity (GO:0016740)	UBE2A, DNMT3B, MBD2, DZIP3, NEK6, UBE2B, KAT6B, DNMT1, CIITA, SETDB2, RNF2, PRMT3, KAT6A, EZH2, RPS6KA5, PRMT8
catalytic activity (GO:0003824)	UBE2A, DNMT3B, DZIP3, USP16, NEK6, UBE2B, KAT6B, DNMT1, HDAC3, HDAC1, CIITA, SETDB2, RNF2, PRMT3, HDAC8, KAT6A, HDAC2, MYSM1, EZH2, RPS6KA5, PRMT8
protein binding (GO:0005515)	UBE2A, DNMT3B, DZIP3, USP16, NEK6, UBE2B, KAT6B, DNMT1, HDAC3, HDAC1, CIITA, SETDB2, RNF2, PRMT3, HDAC8, KAT6A, HDAC2, MYSM1, EZH2, RPS6KA5, PRMT8, MBD2, NCOA6

Supplementary Table S6. Fold regulation and p-values of enzymes with histone deacetylase or methyltransferase activity in MSC from BOS 0p patients vs Control Group.

Functional groups	Gene	Fold Regulation (vs CNT)	pvalue
Histone deacetylase activity	HDAC1	1.9667	0.012195
	HDAC3	1.6235	0.000007
	HDAC8	1.5428	0.008882
	HDAC2	2.0453	0.000845
Methyltransferase activity	DNMT3B	1.5827	0.020132
	DNMT1	1.5812	0.403073
	SETDB2	2.0777	0.161377
	PRMT3	1.7812	0.000519
	EZH2	1.62961	0.306982
	PRMT8	1.9715	0.031952

Supplementary Table S7. Significant deregulated miRNAs in MSC from BOS patients (compared to those from stable LTRs) ($p < 0.05$).

miRNA	P value	Mean1- stable LTRs	Mean2- BOS	Difference	SE of difference	t ratio	df
miR-98	0.011517	1	570118	-570117	158849	3.58905	6
let-7b#	0.038835	1	12819.9	-12818.9	4866.14	2.63431	6
let-7a#	0.039793	1	4753.21	-4752.21	1816.51	2.61612	6
miR-500	0.036645	1	70.872	-69.872	26.0934	2.67777	6
miR-450b-5p	0.03461	1	36.5338	-35.5338	13.0604	2.72072	6
miR-624	0.034554	1	31.0294	-30.0294	11.0324	2.72193	6
miR-16-1#	0.027992	1	24.6085	-23.6085	8.19232	2.88178	6
miR-26a-2#	0.035177	1	21.6069	-20.6069	7.6083	2.70847	6
miR-369-5p	0.041318	1	19.1689	-18.1689	7.02021	2.58809	6
miR-369-3p	0.048489	1	14.9525	-13.9525	5.64989	2.46952	6
miR-126#	0.038298	1	14.2463	-13.2463	5.00859	2.64473	6
miR-7#	0.044091	1	13.7334	-12.7334	5.01347	2.53983	6
miR-576-3p	0.025429	1	13.2402	-12.2402	4.14142	2.95556	6
miR-590-3P	0.02867	1	12.0835	-11.0835	3.87061	2.86351	6
miR-656	0.034865	1	11.6355	-10.6355	3.91704	2.71518	6
miR-301b	0.044478	1	11.0474	-10.0474	3.96603	2.53336	6
miR-579	0.019312	1	10.4067	-9.40669	2.96713	3.17029	6
miR-29b	0.009048	1	9.68861	-8.68861	2.29108	3.79236	6
miR-199b	0.008477	1	7.97715	-6.97715	1.81308	3.84824	6
miR-101	0.048433	1	7.70172	-6.70172	2.71283	2.47038	6
miR-542-3p	0.043178	1	7.52793	-6.52793	2.5546	2.55536	6
miR-455	0.026115	1	7.43916	-6.43916	2.19389	2.93504	6
miR-18a	0.025721	1	7.34824	-6.34824	2.15432	2.94674	6
miR-10b	0.007149	1	7.10499	-6.10499	1.52767	3.99626	6

let-7i#	0.027706	1	6.84317	-5.84317	2.0221	2.88965	6
miR-144#	0.033301	1	6.58335	-5.58335	2.03047	2.74978	6
miR-590-5p	0.016849	1	6.45136	-5.45136	1.66263	3.27876	6
miR-379	0.029727	1	5.96458	-4.96458	1.75062	2.83589	6
miR-15a#	0.009968	1	5.86073	-4.86073	1.31013	3.71011	6
miR-331-5p	0.015028	1	5.65721	-4.65721	1.38164	3.37079	6
miR-381	0.031337	1	5.50338	-4.50338	1.61077	2.79579	6
miR-29c	0.032274	1	5.39161	-4.39161	1.58343	2.77347	6
miR-487a	0.04072	1	5.23061	-4.23061	1.62782	2.59894	6
miR-889	0.036123	1	5.17249	-4.17249	1.55196	2.68853	6
miR-154#	0.036646	1	5.08779	-4.08779	1.52658	2.67774	6
miR-1227	0.011114	1	5.05771	-4.05771	1.12131	3.61873	6
miR-28	0.00911	1	4.83541	-3.83541	1.0129	3.78657	6
miR-185	0.008718	1	4.69541	-3.69541	0.966328	3.82418	6
miR-589	0.022105	1	4.67657	-3.67657	1.19984	3.06422	6
miR-570	0.027173	1	4.41485	-3.41485	1.17569	2.90455	6
miR-130b	0.014096	1	4.24412	-3.24412	0.947803	3.42278	6
miR-1248	0.037875	1	4.11928	-3.11928	1.17574	2.65303	6
miR-497	0.041206	1	3.96628	-2.96628	1.14524	2.5901	6
miR-625	0.038486	1	3.77802	-2.77802	1.05186	2.64106	6
miR-199a	0.028153	1	3.75419	-2.75419	0.957175	2.87742	6
miR-106b	0.037887	1	3.69571	-2.69571	1.01618	2.65278	6
miR-16	0.023453	1	3.63919	-2.63919	0.874445	3.01813	6
miR-339-3p	0.047469	1	3.56397	-2.56397	1.03169	2.48521	6
miR-150	0.000162	1	3.47924	-2.47924	0.297404	8.33627	6
miR-708	0.031803	1	3.44824	-2.44824	0.879202	2.78461	6
miR-10a	0.012259	1	3.3818	-2.3818	0.67334	3.53729	6
miR-942	0.010086	1	3.24606	-2.24606	0.607008	3.70021	6

miR-200c	0.022523	1	3.21972	-2.21972	0.727865	3.04963	6
miR-30b	0.033886	1	2.87222	-1.87222	0.684125	2.73666	6
miR-151-3p	0.042699	1	2.55381	-1.55381	0.606097	2.56364	6
miR-758	0.049001	1	2.50704	-1.50704	0.612176	2.46178	6
miR-25	0.034763	1	2.49583	-1.49583	0.550467	2.71738	6
miR-345	0.049837	1	2.38858	-1.38858	0.566927	2.44932	6
miR-103	0.022021	1	2.27928	-1.27928	0.417085	3.0672	6
miR-324-5p	0.023888	1	1.85113	-0.85113	0.283342	3.00388	6