

## miR-145-5p and miR-203a-5p overcome imatinib resistance in myelogenous leukemic cells through metabolic reprogramming

Priyanka Singh<sup>1</sup>, Sonu Kumar Gupta<sup>1</sup>, Villayat Ali<sup>1</sup>, Ravindresh Chhabra<sup>1\*</sup> & Malkhey Verma<sup>1,2\*</sup>

<sup>1</sup>Department of Biochemistry, School of Basic Sciences, Central University of Punjab, Bathinda-151 401, Punjab, India

<sup>2</sup>School of Biotechnology, Institute of Science, Banaras Hindu University, Varanasi-221 005, Uttar Pradesh, India

Received 14 September 2022; revised 14 December 2022

### Supplementary Data

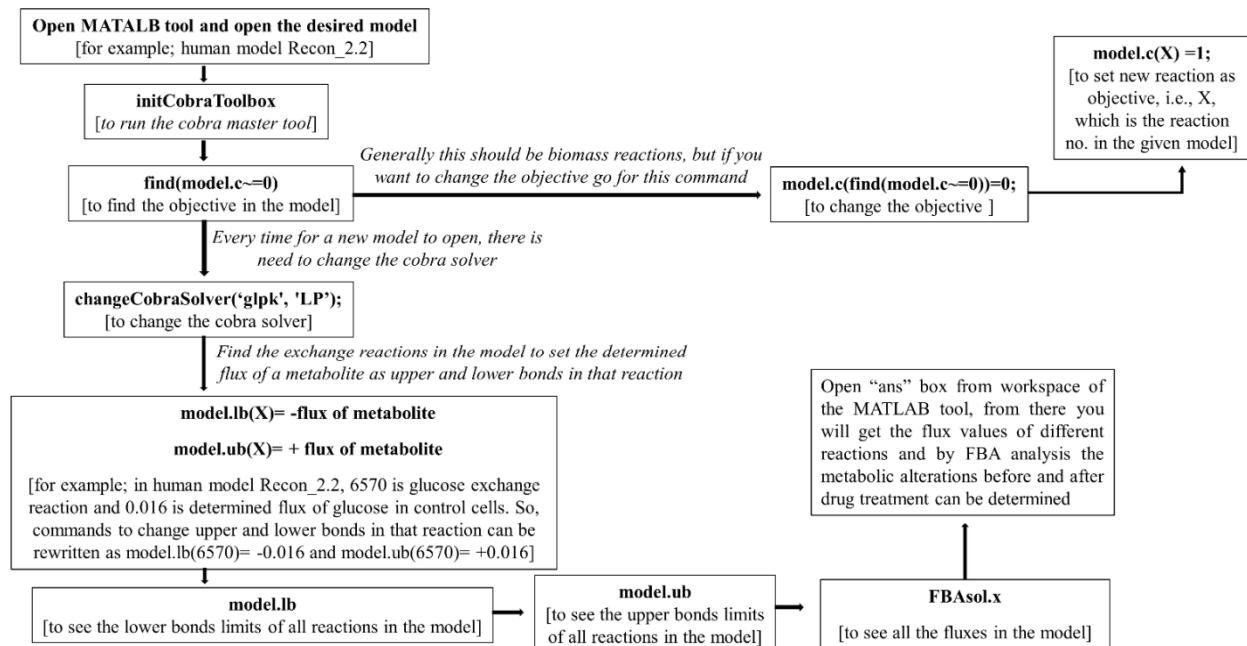


Fig. S1 — A schematic illustration of the search strategy used to determine the flux balance analysis (FBA) in the human model Recon\_2.2

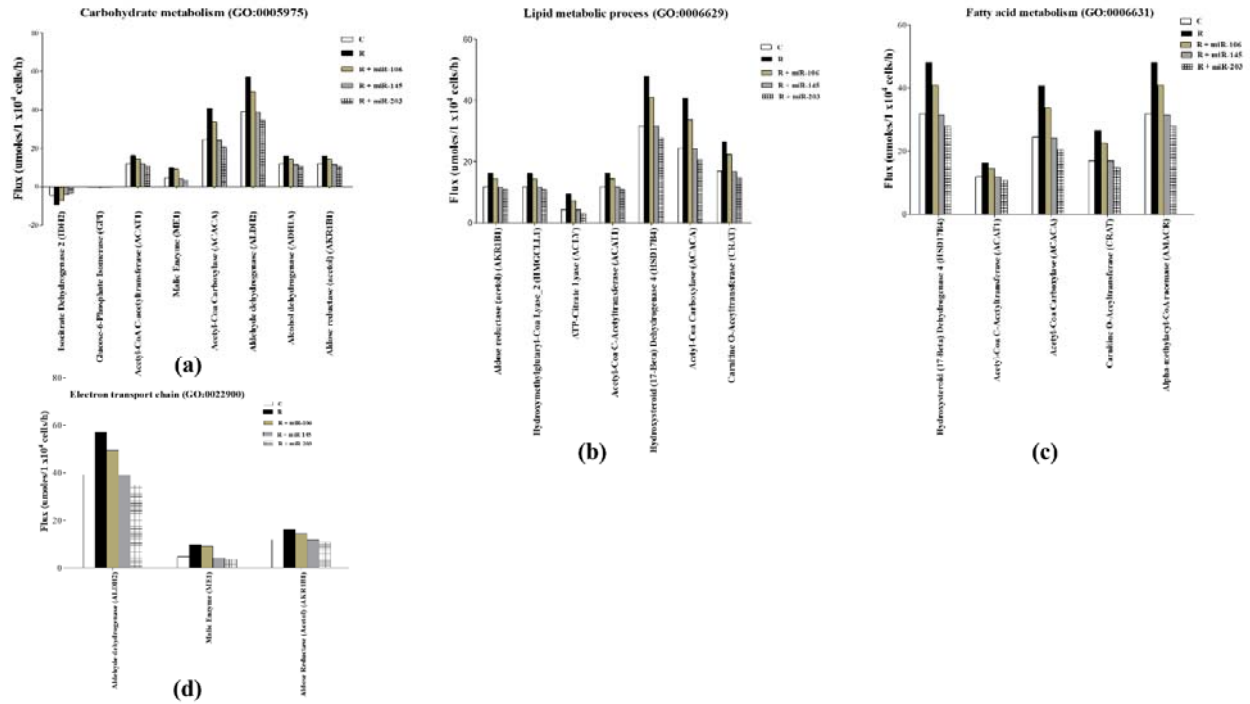


Fig. S2 — Calculated flux of enzymes regulating (A) Carbohydrate metabolism; (B) Lipid metabolic process; (C) Fatty acid metabolism; and (D) Electron Transport Chain. C: control (normal K562 cells), R: imatinib-resistant K562 cells (IR-K562 cells), R + miR-106: IR-K562 cells transfected with miR-106b-5p, R + miR-145: IR-K562 cells transfected with miR-145-5p, and R + miR-203: IR-K562 cells transfected with miR-203a-5p

Table S1 — Sequences of the primers for RT-PCR analysis

	Forward Primer (5'-3')	Reverse Primer (5'-3')
GAPDH	AGCCACATCGCTCAGACAC	GCCCAATACGACCAAATCC
miR-106b-5p RT FP	ACACTCCAGCTGGGTAAAGTGCTGACAGT	GTGTCGTGGAGTTCGGCAATTC
miR-145-5p RT FP	ACACTCCAGCTGGGGTCCAGTTTTCCAGGA	GTGTCGTGGAGTTCGGCAATTC
miR-203a-5p RT FP	ACACTCCAGCTGGGGTCAAATGTTTAGGAC	GTGTCGTGGAGTTCGGCAATTC
ALDH2	ATGTCTCCGGTATTATGCC	ATTCCACGGAATGATCTGC
ACACA	ATCTTGAGGGCTAGGTCTTTTGGGA	CCAGAGGTTGGCCAAGGGA
ACAT1	GAACAGAGGATCAACACCAT	TGCTGCTTACTTCTGGTAT

Table S2 — The complete list of changes in the flux values of enzymes and metabolic reactions in the resistant cells as revealed by the FBA analysis

Reaction no.	Enzymes/Metabolisms	Metabolic reactions	Control	Resistant	R + miR-106b-5p	R + miR-145-5p	R + miR-203a-5p
11	2-Aminoacrylate hydrolysis	water{cytoplasm} + "2-aminoacrylic acid" -> ammonium{cytoplasm} + pyruvate{cytoplasm}	16.21	25.76	21.57	16.04	13.87
71	Aminoacetone:oxygen oxidoreductase(deaminating) (flavin-containing)	water{cytoplasm} + dioxygen{cytoplasm} + ammonioacetone{cytoplasm} -> "hydrogen peroxide"{cytoplasm} + ammonium{cytoplasm} + methylglyoxal{cytoplasm}	17.18	26.73	22.54	17.01	14.84

89	acetyl-CoA C-acetyltransferase, mitochondrial_3	2 * acetyl-CoA(4-){peroxisome} ->acetoacetyl-CoA(4-){peroxisome} + "coenzyme A (4-)"{peroxisome}	11.85	16.28	14.34	11.77	10.76
96	acetyl-CoA carboxylase	ATP(4-){cytoplasm} + hydrogencarbonate{cytoplasm} + acetyl-CoA(4-){cytoplasm} -> ADP(3-){cytoplasm} + proton{cytoplasm} + hydrogenphosphate{cytoplasm} + malonyl-CoA(5-){cytoplasm}	24.51	40.72	33.62	24.22	20.54
109	ATP-Citrate lyase	ATP(4-){cytoplasm} + "coenzyme A(4-)"{cytoplasm} + citrate(3-){cytoplasm} -> ADP(3-){cytoplasm} + hydrogenphosphate{cytoplasm} + acetyl-CoA(4-){cytoplasm} + oxaloacetate(2-){cytoplasm}	4.37	9.49	7.25	4.28	3.12
133	acetone monooxygenase	dioxygen{cytoplasm} + proton{cytoplasm} + NADPH(4-){cytoplasm} + acetone{cytoplasm} -> water{cytoplasm} + NADP(3-){cytoplasm} + hydroxyacetone	11.78	16.21	14.27	11.69	10.69
137	Acetoacetate decarboxylation (irreversible), mitochondrial	proton{mitochondrion} + acetoacetate{mitochondrion} -> "carbon dioxide"{mitochondrion} + acetone{mitochondrion}	11.78	16.21	14.27	11.69	10.69
138	adenylate kinase	ATP(4-){cytoplasm} + AMP(2-){cytoplasm} = 2 * ADP(3-){cytoplasm}	11.45	16.87	14.28	12.35	10.64
203	alcohol dehydrogenase (L-1,2-propanediol)	NAD(1-){cytoplasm} + (S)-propane-1,2-diol -> proton{cytoplasm} + NADH(2-){cytoplasm} + (S)-lactaldehyde{cytoplasm}	11.78	16.21	14.27	11.69	10.69
204	alcohol dehydrogenase (L-lactaldehyde)	NAD(1-){cytoplasm} + (S)-lactaldehyde{cytoplasm} -> proton{cytoplasm} + NADH(2-){cytoplasm} + methylglyoxal{cytoplasm}	11.78	16.21	14.27	11.69	10.69
209	aldehyde dehydrogenase (pristanal, NAD)	water{cytoplasm} + NAD(1-){cytoplasm} + pristanal{cytoplasm} -> 2 * proton{cytoplasm} + NADH(2-){cytoplasm} + "pristanic acid"{cytoplasm}	39.16	57.19	49.28	38.83	34.75
220	aldose reductase (acetol)	proton{cytoplasm} + NADPH(4-){cytoplasm} + hydroxyacetone -> NADP(3-){cytoplasm} + (S)-propane-1,2-diol	11.78	16.21	14.27	11.69	10.69
221	alpha-methylacyl-CoA racemase (reductase)	0.5 * dioxygen{peroxisome} + 3alpha,7alpha-dihydroxy-5beta-cholestan-26-oyl-CoA{peroxisome} -> 3alpha,7alpha-dihydroxy-5beta-cholest-24-enoyl-CoA{peroxisome} + water{peroxisome}	31.79	48.00	40.90	31.50	27.82
232	L-2-amino-3-oxobutanoate decarboxylation,	proton{mitochondrion} + "L-2-amino-3-oxobutanoic acid" -	17.18	26.73	22.54	17.01	14.84

	mitochondrial (spontaneous)	>"carbon dioxide"{mitochondrion} +					
342	catalase A, peroxisomal	ammonioacetone{mitochondrion} 2 * "hydrogen peroxide"{peroxisome} -> dioxygen{peroxisome} + 2 * water{peroxisome}	32.01	47.78	40.86	31.72	28.14
402	carnitine O-acetyltransferase, mitochondrial_2	coenzyme A(4-){mitochondrion} + O-acetyl-L- carnitine{mitochondrion} = acetyl-CoA(4-){mitochondrion} + (R)-carnitine{mitochondrion}	17.00	26.55	22.36	16.83	14.66
403	carnitine O-acetyltransferase, reverse direction, peroxisomal	acetyl-CoA(4-){peroxisome} + (R)-carnitine{peroxisome} = "coenzyme A(4-)"{peroxisome} + O-acetyl-L-carnitine{peroxisome}	40.24	62.91	52.98	39.83	34.69
404	carnitine O-acetyltransferase	acetyl-CoA(4-){cytoplasm} + (R)- carnitine{cytoplasm} = "coenzyme A(4-)"{cytoplasm} + O-acetyl-L-carnitine{cytoplasm}	-23.24	-36.36	-30.61	-23.01	-20.03
679	fatty acyl-CoA synthase	3 * proton{cytoplasm} + 2 * NADPH(4-){cytoplasm} + malonyl-CoA(5-){cytoplasm} + octanoyl-CoA(4-){cytoplasm} - >water{cytoplasm} + 2 * NADP(3-) {cytoplasm} + "carbon dioxide"{cytoplasm} + "coenzyme A(4-)"{cytoplasm} + decanoyl- CoA(4-){cytoplasm}	3.11	5.13	4.24	3.07	2.61
873	glycine hydroxymethyltransferase, reversible	(6S)-5,6,7,8-tetrahydrofolate(2-) {cytoplasm} + L- serine{cytoplasm} = water{cytoplasm} + glycine{cytoplasm} + (6R)-5,10- methylenetetrahydrofolate(2-) {cytoplasm}	-17.43	-26.98	-22.80	-17.26	-15.09
934	glycine C-acetyltransferase	acetyl-CoA(4-){mitochondrion} + glycine{mitochondrion} = "coenzyme A(4-) "{mitochondrion} + "L-2-amino- 3-oxobutanoic acid"	17.18	26.73	22.54	17.01	14.84
948	glycine N-methyltransferase	S-adenosyl-L- methionine{cytoplasm} + glycine{cytoplasm} -> proton{cytoplasm} + S-adenosyl- L-homocysteine{cytoplasm} + sarcosine{cytoplasm}	46.99	70.53	60.21	46.57	41.23
974	carboxylic acid dissociation	water{cytoplasm} + "carbon dioxide"{cytoplasm} = proton{cytoplasm} + hydrogencarbonate{cytoplasm}	24.58	40.79	33.69	24.29	20.60
1016	hydroxymethylglutaryl-CoA lyase_2	(3S)-3-hydroxy-3-methylglutaryl- CoA{peroxisome} -> acetyl- CoA(4-){peroxisome} + acetoacetate{peroxisome}	11.78	16.21	14.27	11.69	10.69
1033	hydroxysteroid (17-beta) dehydrogenase 4	water{peroxisome} + NADP(3-) {peroxisome} + 3alpha,7alpha- dihydroxy-5beta-cholest-24-enoyl- CoA{peroxisome} - >proton{peroxisome} + NADPH(4-) {peroxisome} + 3alpha,7alpha-	31.79	48.00	40.90	31.50	27.82

1055	Isocitrate dehydrogenase (NADP <sup>+</sup> )_2	dihydroxy-24-oxo-5beta-cholestan-26-oyl-CoA NADP(3-){mitochondrion} + isocitrate(3-){mitochondrion} = NADPH(4-){mitochondrion} + 2-oxoglutarate(2-){mitochondrion} + "carbon dioxide" {mitochondrion}	-4.37	-9.49	-7.25	-4.28	-3.12
1099	L-lactate dehydrogenase_2	NAD(1-){mitochondrion} + (S)-lactate {mitochondrion} = proton {mitochondrion} + NADH(2-){mitochondrion} + pyruvate {mitochondrion}	7.10	6.41	6.71	7.11	7.27
1181	malic enzyme (NAD), mitochondrial	NAD(1-){mitochondrion} + (S)-malate(2-){mitochondrion} -> "carbon dioxide" {mitochondrion} + NADH(2-){mitochondrion} + pyruvate {mitochondrion}	4.68	9.80	0.06	0.06	3.42
1254	methylenetetrahydrofolate dehydrogenase (NAD)	NAD(1-){cytoplasm} + (6R)-5,10-methylenetetrahydrofolate(2-){cytoplasm} = NADH(2-){cytoplasm} + (6R)-5,10-methenyltetrahydrofolate {cytoplasm}	-17.44	-26.99	-22.80	-17.27	-50.73
1510	glucose-6-phosphate isomerase	D-glucopyranose 6-phosphate(2-){cytoplasm} = "D-fructose 6-phosphate(2-)"	-0.16	-0.16	-0.16	-0.16	-0.16
1778	Sarcosine dehydrogenase (m)	FAD {mitochondrion} + sarcosine {mitochondrion} + (6S)-5,6,7,8-tetrahydrofolate(2-){mitochondrion} -> FADH2(2-){mitochondrion} + glycine {mitochondrion} + (6R)-5,10-methylenetetrahydrofolate(2-){mitochondrion}	46.99	70.53	60.21	46.57	41.23
1783	peroxisomal thiolase 2	coenzyme A(4-){peroxisome} + 3alpha,7alpha-dihydroxy-24-oxo-5beta-cholestan-26-oyl-CoA ->chenodeoxycholoyl-CoA {peroxisome} + propionyl-CoA(4-){peroxisome}	31.79	48.00	40.90	31.50	27.82
1793	L-Serine hydro-lyase	L-serine {cytoplasm} -> water {cytoplasm} + "2-aminoacrylic acid"	16.21	25.76	21.57	16.04	13.87
2008	2-Oxopropanal:NADP <sup>+</sup> oxidoreductase Pyruvate metabolism EC:1.2.1.49	water {cytoplasm} + NADP(3-){cytoplasm} + methylglyoxal {cytoplasm} = 2 * proton {cytoplasm} + pyruvate {cytoplasm} + NADPH(4-){cytoplasm}	28.96	42.94	36.81	28.70	25.53
2066	glycine synthase Nitrogen metabolism EC:2.1.2.10	carbon dioxide {mitochondrion} + NADH(2-){mitochondrion} + ammonium {mitochondrion} + (6R)-5,10-methylenetetrahydrofolate(2-){mitochondrion} -> NAD(1-){mitochondrion} + glycine {mitochondrion} + (6S)-5,6,7,8-tetrahydrofolate(2-){mitochondrion}	29.91	43.89	37.76	29.65	26.48

3888	Complex II reaction for respiratory chain	FADH <sub>2</sub> (2-){mitochondrion} + "coenzyme Q10"{mitochondrion} -> FAD{mitochondrion} + ubiquinol-10{mitochondrion}	47.74	71.28	60.96	47.31	41.98
3919	10-Formyltetrahydrofolate mitochondrial transport <i>via</i> diffusion	10-formyltetrahydrofolate(2-){cytoplasm} = 10-formyltetrahydrofolate(2-){mitochondrion}	-17.08	-26.64	-22.45	-16.91	-14.75
3982	Aminoacetone transport (mitochondrial)	ammonioacetone{mitochondrion} -> ammonioacetone{cytoplasm}	17.18	26.73	22.54	17.01	14.84
4110	ATP synthase (four protons for one ATP)	4 * proton{"mitochondrial intermembrane space"} + ADP(3-){mitochondrion} + hydrogenphosphate{mitochondrion} -> 3 * proton{mitochondrion} + water{mitochondrion} + ATP(4-){mitochondrion}	72.20	107.50	92.03	71.56	63.55
4111	ADP/ATP transporter, mitochondrial	ADP(3-){cytoplasm} + ATP(4-){mitochondrion} -> ATP(4-){cytoplasm} + ADP(3-){mitochondrion}	71.57	107.60	91.41	71.68	62.94
4208	carnitine-acetylcarnitine carrier, peroxisomal	(R)-carnitine{cytoplasm} + O-acetyl-L-carnitine{peroxisome} -> O-acetyl-L-carnitine{cytoplasm} + (R)-carnitine{peroxisome}	40.24	62.91	52.98	39.83	34.69
4236	ubiquinol-6 cytochrome c reductase, Complex III	2 * proton{mitochondrion} + 2 * "ferricytochrome c" + ubiquinol-10{mitochondrion} -> 4 * proton{"mitochondrial intermembrane space"} + "coenzyme Q10"{mitochondrion} + 2 * "ferrocytochrome c"	48.13	71.67	61.35	47.70	42.37
4256	D-alanine transport <i>via</i> proton symport	proton{"extracellular space"} + D-alanine{"extracellular space"} = proton{cytoplasm} + D-alanine{cytoplasm}	0.02	0.02	0.02	0.02	0.01
4493	glucose transport <i>via</i> sodium symport	sodium(1+){"extracellular space"} + D-glucopyranose{"extracellular space"} = sodium(1+){cytoplasm} + D-glucopyranose{cytoplasm}	0.02	0.02	0.02	0.02	0.01
4507	L-glutamate reversible transport <i>via</i> proton symport, mitochondrial	proton{cytoplasm} + L-glutamate(1-){cytoplasm} -> proton{mitochondrion} + L-glutamate(1-){mitochondrion}	4.37	9.49	7.25	4.28	3.12
4523	glycine passive transport to mitochondria	glycine{cytoplasm} = glycine{mitochondrion}	-59.72	-87.69	-75.43	-59.21	-52.87
4931	5,6,7,8-Tetrahydrofolate transport, diffusion, mitochondrial	(6S)-5,6,7,8-tetrahydrofolate(2-){cytoplasm} = (6S)-5,6,7,8-tetrahydrofolate(2-){mitochondrion}	17.08	26.64	22.45	16.91	14.75
6645	Ammonia exchange	ammonium{"extracellular space"} = ammonium{boundary}	8.57	18.81	14.32	8.21	6.06
7176	uptake of acetaminophen-glutathione-conjugate into the enterocytes	acetaminophen glutathione conjugate{"extracellular space"} = "acetaminophen glutathione conjugate"{cytoplasm}	5.02	10.14	7.90	4.93	3.77