Tumor suppressors p53, p63, and p73 inhibit migrating cancer stem cells by increasing the expression of stem cell suppressing miRNAs

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Abstract

ZEB1/2 has been shown to suppress the expression of the tumor suppressors E-cadherin, TA-p73, INK4b, CDKN1a, and thereby promotes epithelial to mesenchymal transition, invasion, and metastasis. I have recently shown that p53-dependent miRs, such as miR-192, 215, 145, 203, 200b/c, 183, suppress the expression of ZEB1/2. Another recent study showed that ZEB1/2 suppresses the expression of miR-200b/c, miR-203, & miR-183, which in turn, inhibit the expression of stem cell factors, such as Sox-2, Klf-4, and BMI. ZEB1/2, by inhibiting the expression of stem cell factors suppressing miRs, it up regulates the expression of stem cell factors. By up regulating the stem cell factors and down regulating metastasis suppressors, ZEB1/2 promotes both metastasis and migration of cancer stem cells. p53/p63/p73, by down regulating the expression of ZEB1/2 through its target miRNAs, it could activate the expression of stem cell suppressing miRNAs, such as miR-200b/c, miR-203 & miR-183. Thereby, it could inhibit the expression of stem cell factors, such as Sox-2, Klf-4 and BMI-1(an inhibitor of INK4a/ARF). In addition, p53/p63/p73 appears to inhibit the expression of other EMT inducers, such as Snail1/2 and Twist, expression through its target miRs. Remarkably, bioinformatics analysis of stem cell factors suppressing miRNAs, miR-200, miR-183, miR-141, and miR-203, promoters revealed a number of p53/p63/p73 responsive elements, indicating that they could be

direct transcriptional targets of p53/TA-p63/p73. Together, these data suggest that p53/TA-p63/p73, by suppressing the expression of ZEB1/2, Snail1/2, and Twist through its target miRs, it could inhibit migration, metastasis, and proliferation of cancer stem cells. In conclusion, the data presented here provide mechanistic insights into how TA-p63/p73 and p53 function as metastasis suppressors.

Introduction

ZEB1/2 functions as an activator of epithelial to mesenchymal transition [EMT] and an inhibitor of mesenchymal to epithelial transition [MET]. The EMT promotes invasion, metastasis, self-renewal, and cancer stem cell (CSC) proliferation. ZEB1/2 suppresses the expression of the tumor suppressors E-cadherin, and TA-p73, and thereby promotes EMT, invasion and metastasis. I have recently shown that p53-dependent miRs, such as miR-192, 215, 145, 203, 200b/c, 183, suppress the expression of ZEB1/2 (1).

Results and Discussion

A recent study showed that ZEB1/2 suppresses the expression of tumor suppressor miRNAs, such as miR-200b/c, miR-203, & miR-183 (2) [Figure 1]. Furthermore, this study showed that miR-200b/c, miR-203, let-7, & miR-183 inhibit the expression of stem cell factors, such as Sox-2, Klf-4, and BMI [Figure 2] (2). By inhibiting the expression of stem cell factors suppressing miRs, ZEB1/2 up regulates the expression of stem cell factors. By up regulating the stem cell factors and down regulating metastasis suppressors (E-cadherin & TA-p73), ZEB1/2 promotes both metastasis and migration of cancer stem cells [Figure 3-8]. On the other hand,

p53/TA-p63/p73, by down regulating the expression of ZEB1/2 through its target miRNAs, it could activate the expression of stem cell suppressing miRNAs, such as miR-200b/c, miR-203 & miR-183. Thereby, it could inhibit the expression of stem cell factors, such as Sox-2, Klf-4 and BMI-1(an inhibitor of INK4a/ARF expression) [Figure 9].

In addition, a. miR-203 appears to target the expression of Ihh, SMO, PDGF-D, FZD1, VEGF, SELE, ΔNp63, BMI-1, Stem cell factor (SCDF1), lin-28B (an inhibitor of the tumor suppressor miR, let-7) and RAP1A (increases self-renewal of ESCs); b. miR-200b/c appears target the expression of patched 1, RPS6KB1, BMI-1, and Rac-1; c. miR-183 appears to target the expression of TrCP1, ZEB1, TCF-4, and NFKB1(3); and d. miR-141 appears to target the expression of ZEB1/2, TCF-4, TGF 2, E2F3, CDK6, CCND2, CCNE2, and ESRRB. These data suggest that increased expression of miR-203, miR-183, miR-141, and miR-200b/c will inhibit proliferation, growth, EMT, invasion, metastasis, migration, and survival of cancer stem cells [Figure 3-9].

In addition, it has recently been shown that *ZEB1*$^{-/-}$ cells express increased levels of INK4b and CDKN1a, suggesting that ZEB1 could be a negative regulator of INK4b and CDKN1a (4). Interestingly, *ZEB*$^{-/-}$ cells have been shown to undergo senescence, suggesting that increased expression of INK4b may promote senescence (4). This data suggests that p53/TA-p73/p63, by inhibiting the expression of ZEB1/2, it could activate a senescence program.

Remarkably, bioinformatics analysis of miR-203, miR-183, miR-141, and miR-200b/c promoters indicated that these promoters contain a number of p53/p63/p73-REs (Table 1-5). In addition, I have recently shown that let-7 promoter contains p53/p63/p73-REs (5). These data suggest that miR-200, miR-183, miR-141, miR-203, and let-7 could be direct transcriptional targets of p53/TA-p63/p73. Together, this study suggests that p53/TA-p63/p73, by suppressing the expression of ZEB1/2, Snail1/2, and Twist through its target miRs, it could inhibit EMT, migration, metastasis, and proliferation of cancer stem cells. In conclusion, this study provides mechanistic insights into how p53/TA-p63/p73 functions as an inhibitor of EMT and a metastasis suppressor (Figure 9).

Legends and figures

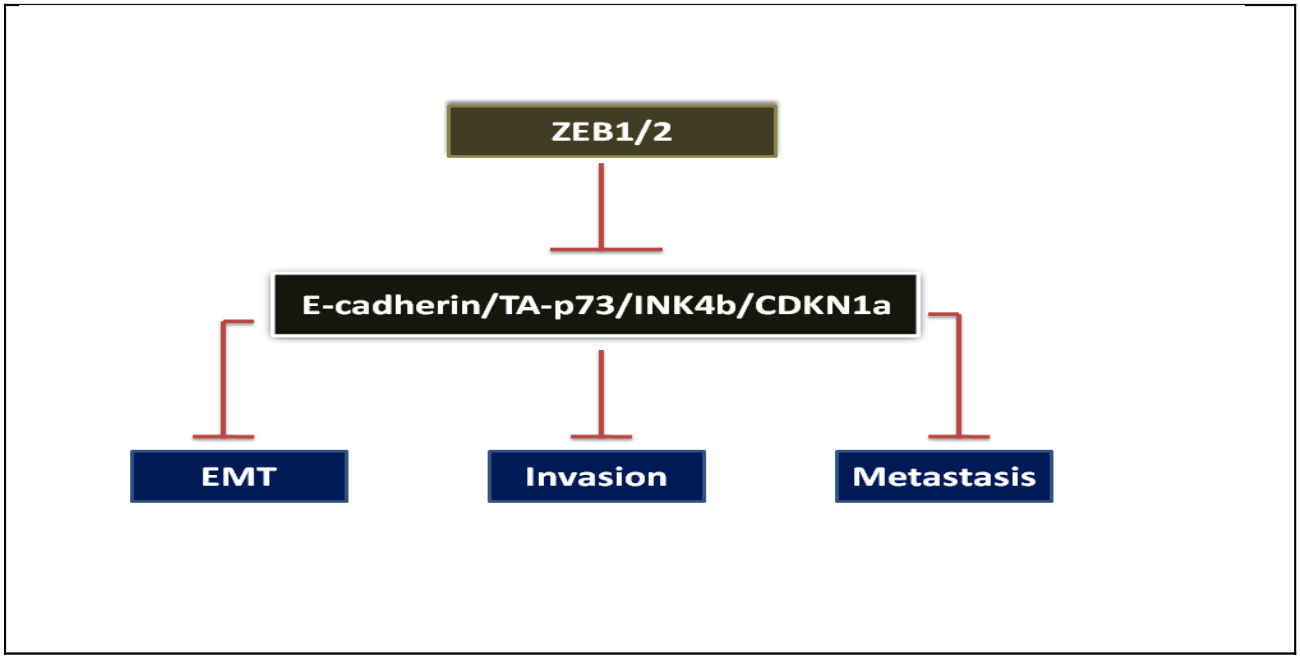
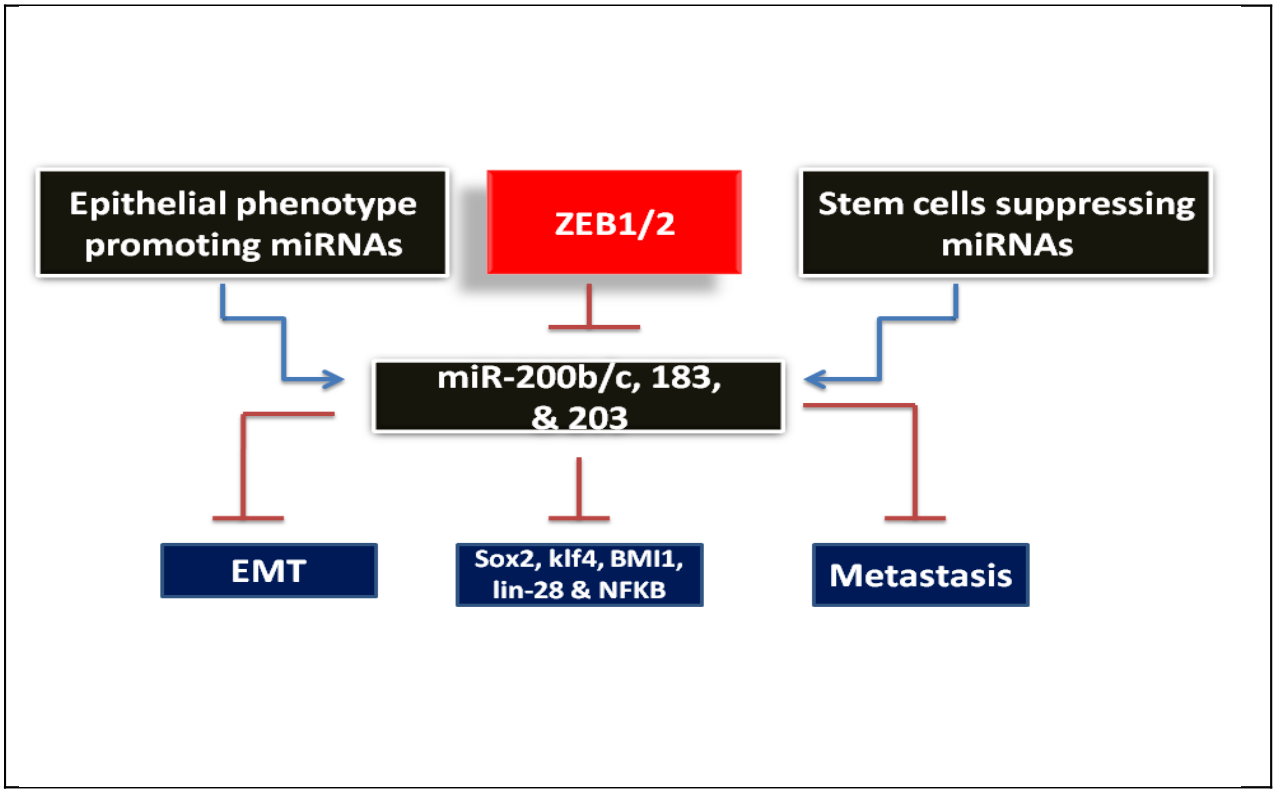


Figure 1 ZEB1/2 inhibits E-cadherin/TA-p73/INK4b/CDKN1a expression to promote migration, invasion and metastasis.



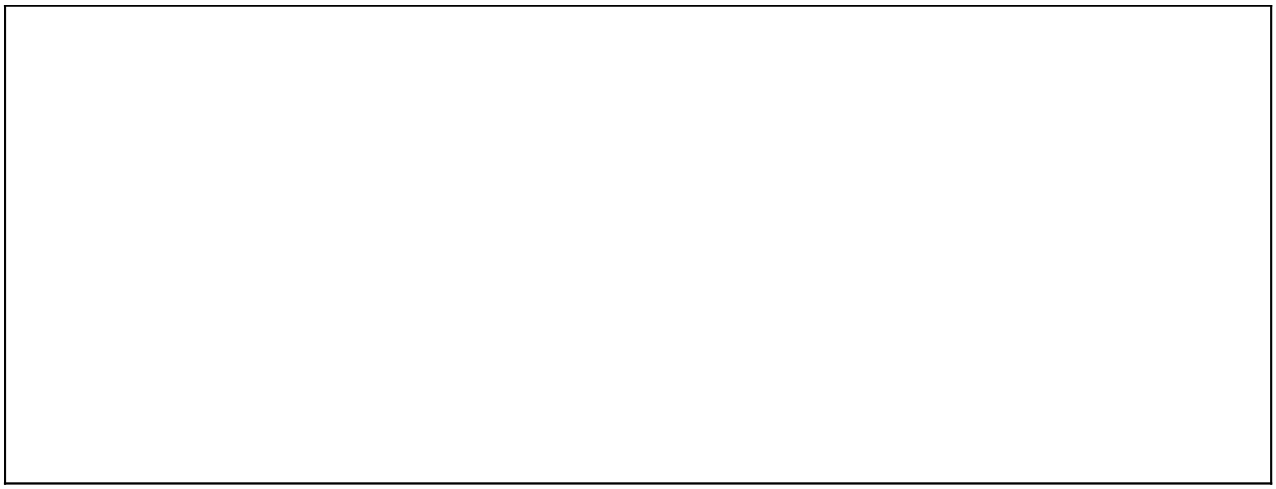


Figure 2 ZEB1/2 suppresses stem cells suppressing/epithelial phenotype promoting miRNAs to increase the expression of stem cell factors.

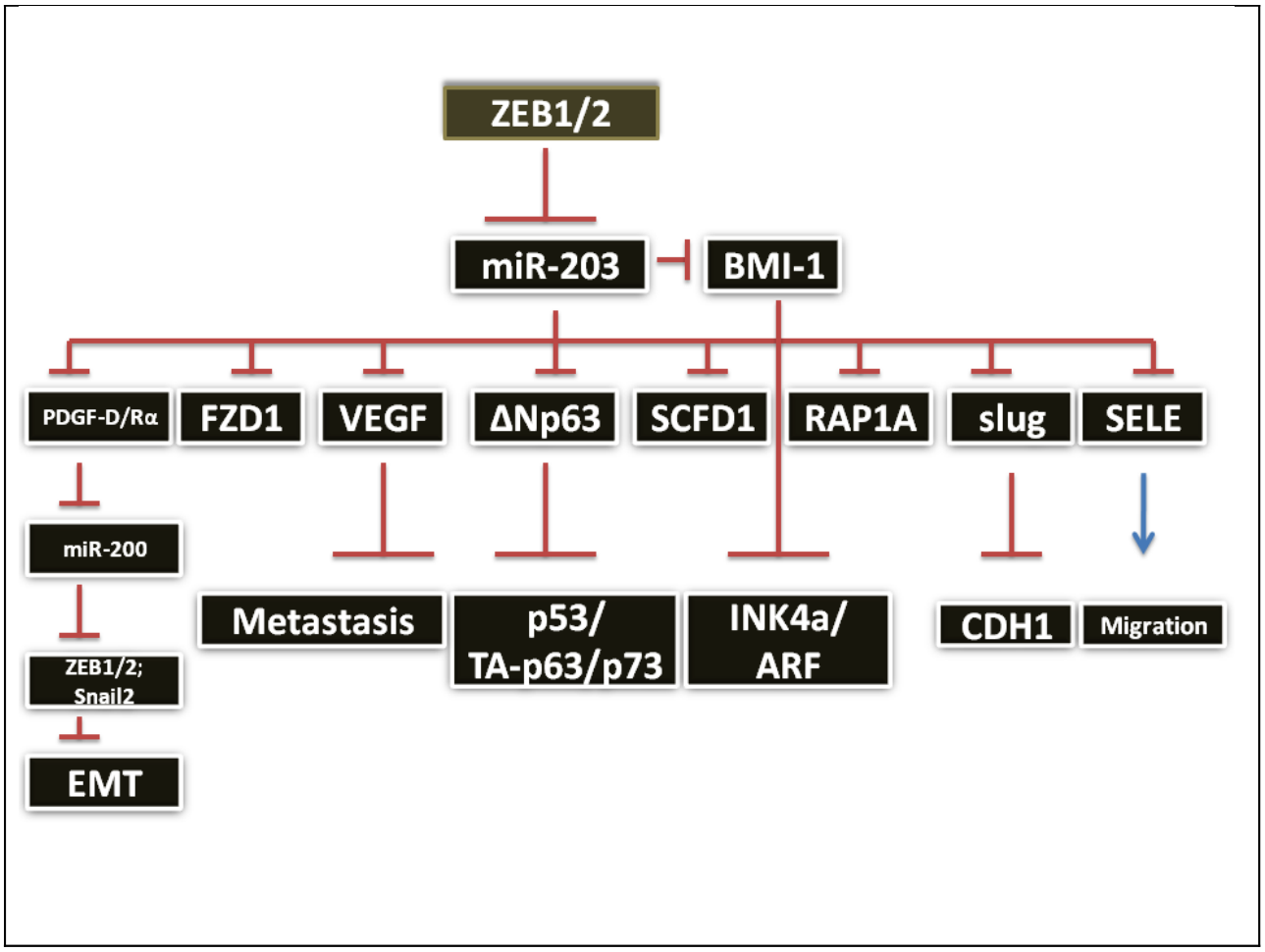


Figure 3 ZEB1/2 inhibits the stem cell suppressing miRNA-203 to promote EMT, invasion, metastasis, and migration of CSCs.

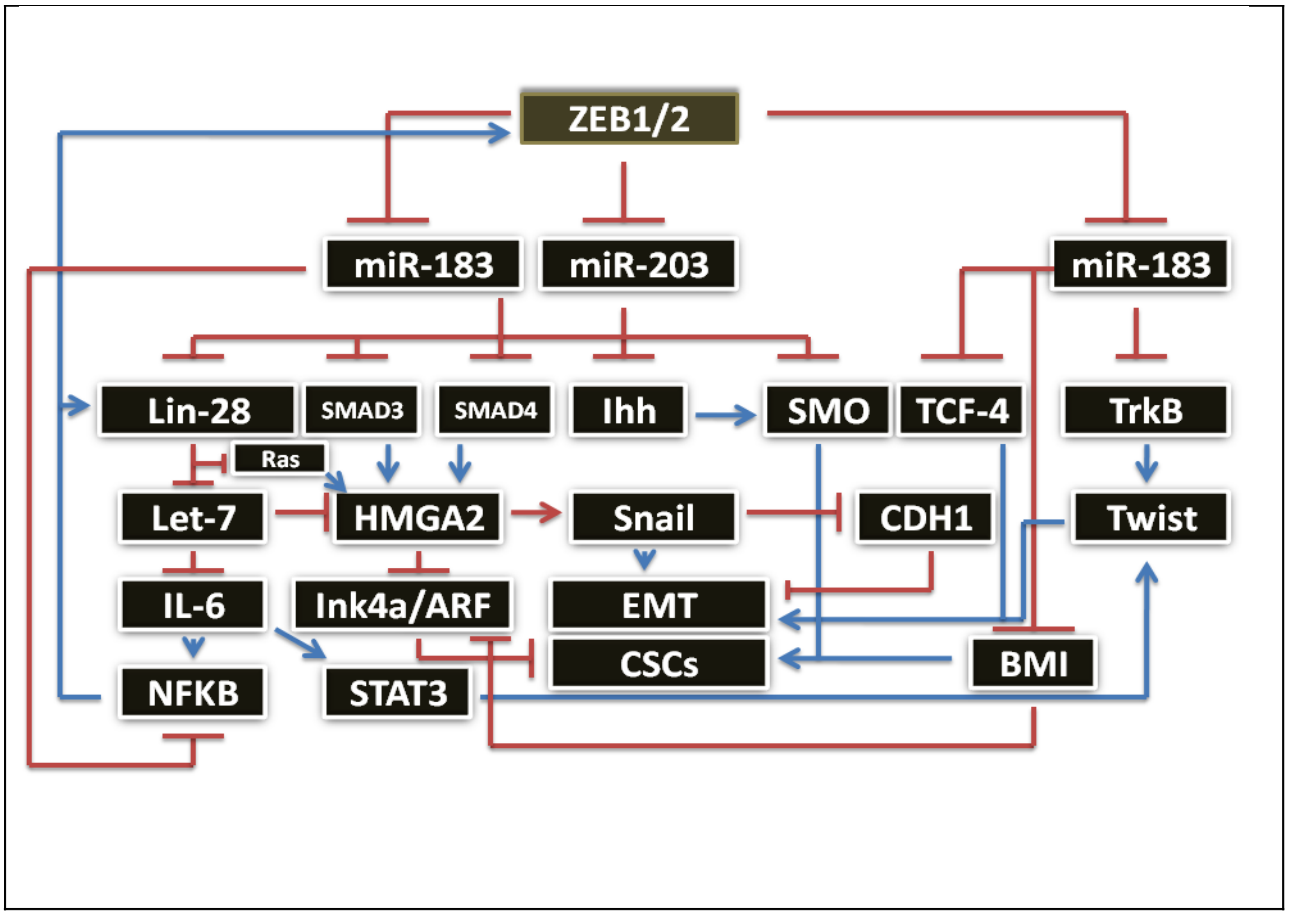


Figure 4 ZEB1/2 inhibits the stem cell suppressing miRNA-183 to promote EMT, invasion, metastasis, and migration of CSCs.

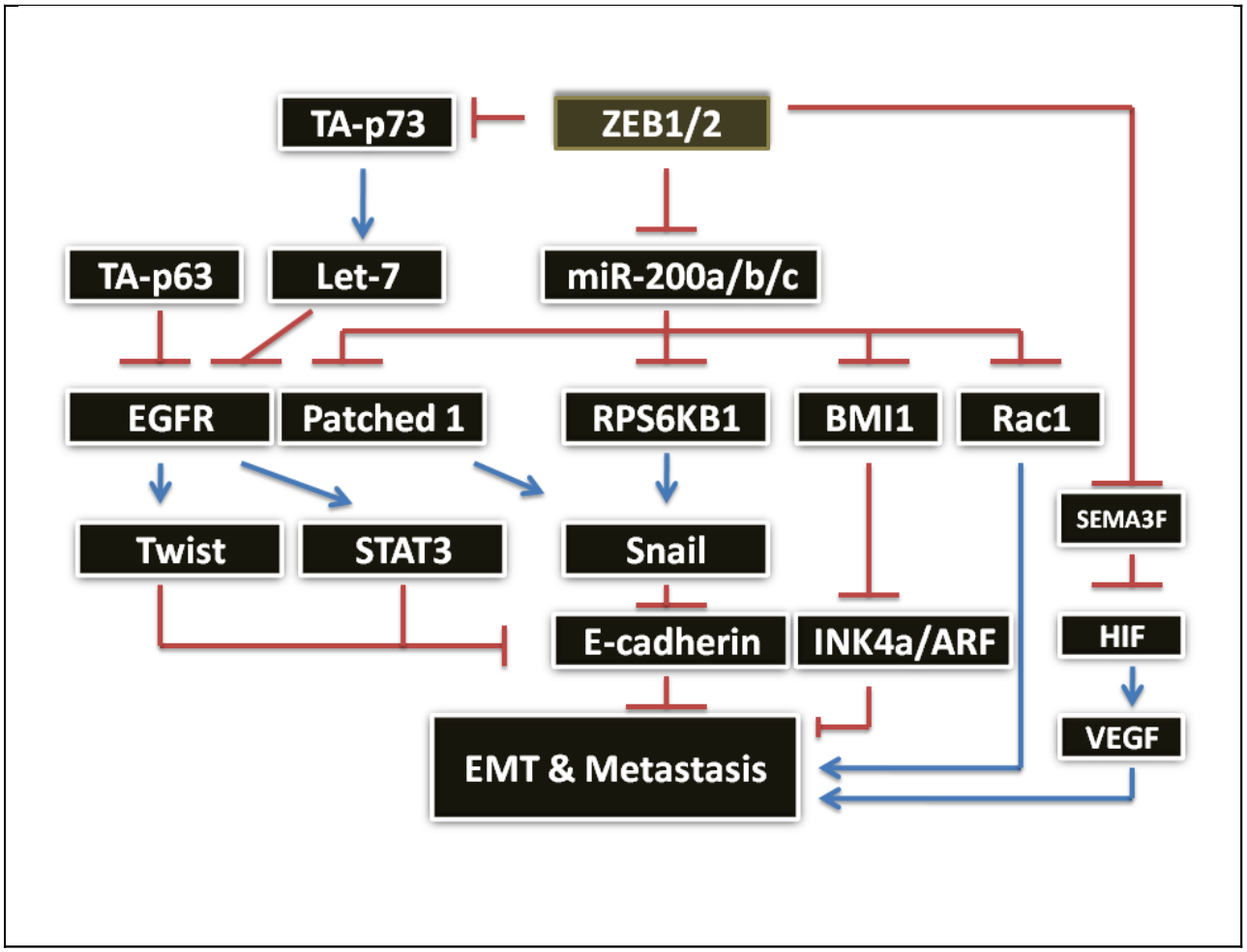


Figure 5 ZEB1/2 inhibits the stem cell suppressing miRNA-200 to promote EMT, metastasis, and migration of CSCs.

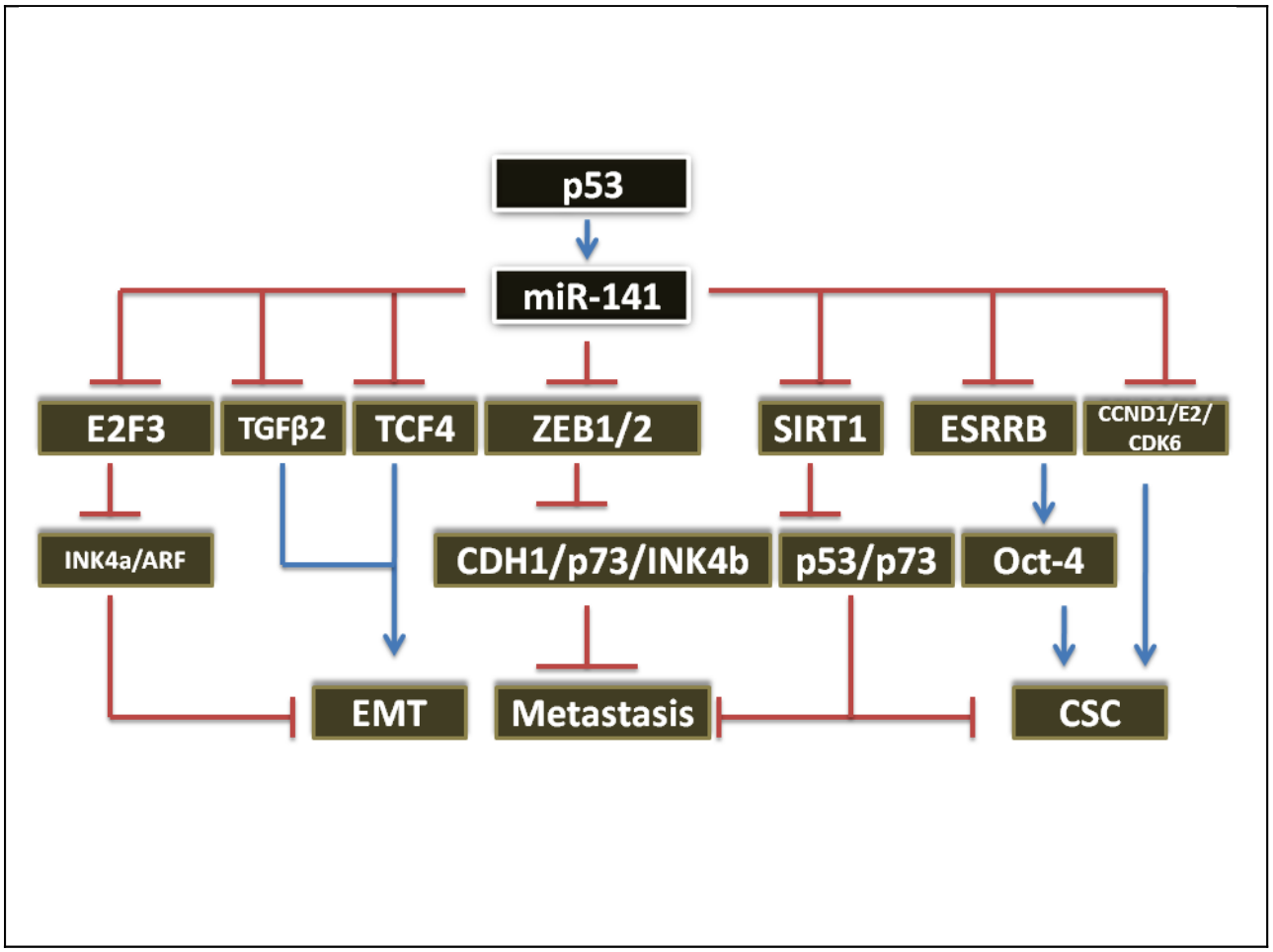


Figure 6 p53 increases the stem cell suppressing miRNA-141 to inhibit metastasis and CSCs proliferation.

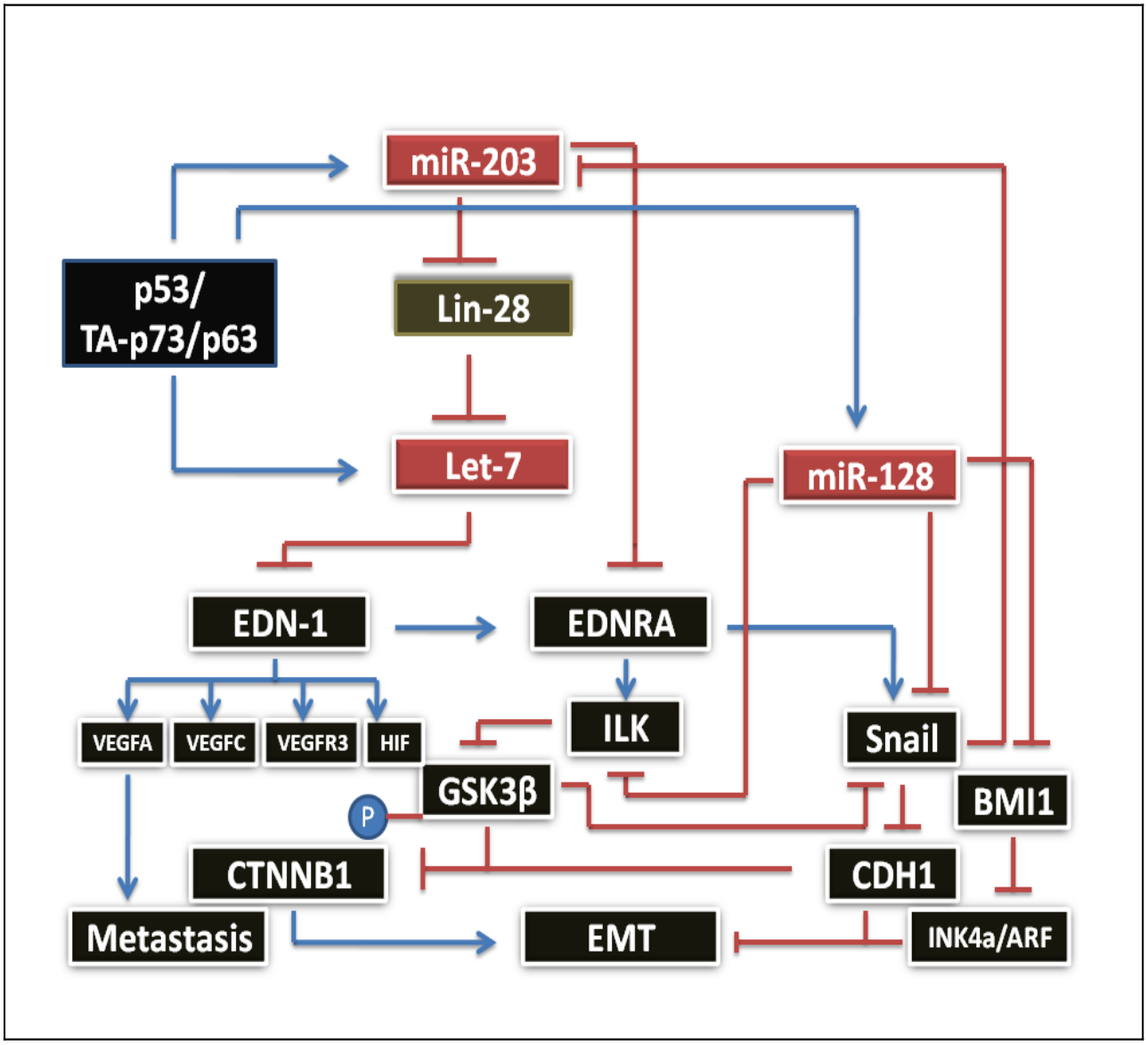


Figure 7 p53/TA-p73/p63 increases stem cell suppressing miRNAs to inhibit EDN1-EDNRA pathway, EMT, and metastasis.

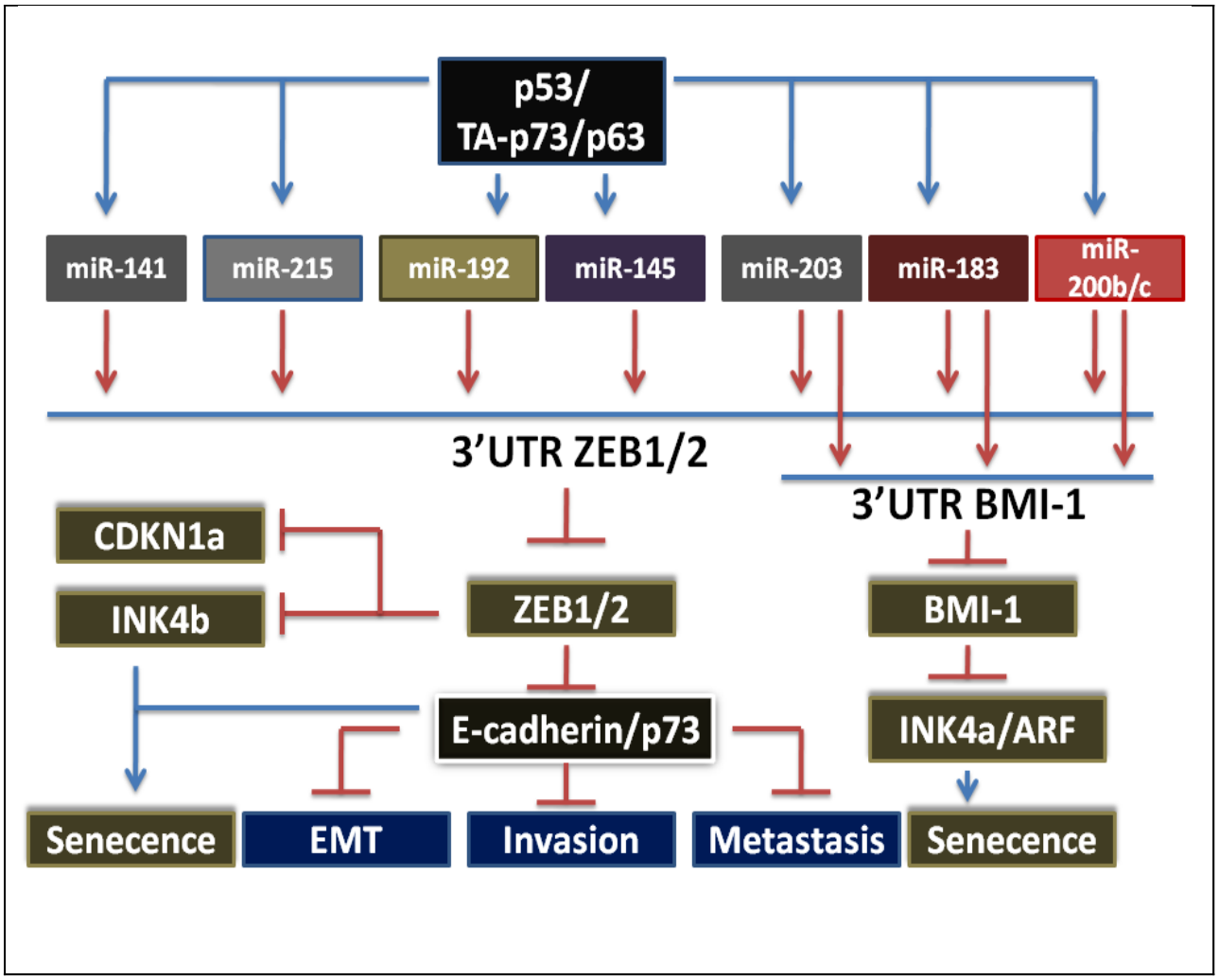


Figure 8 p53/TA-p73/p63 increases Stem cell suppressing miRNAs to inhibit ZEB1/ZEB2 and to activate INK4a,b/ARF/CDKN1a expression (Red arrow indicates inhibition; Blue arrow indicates activation).

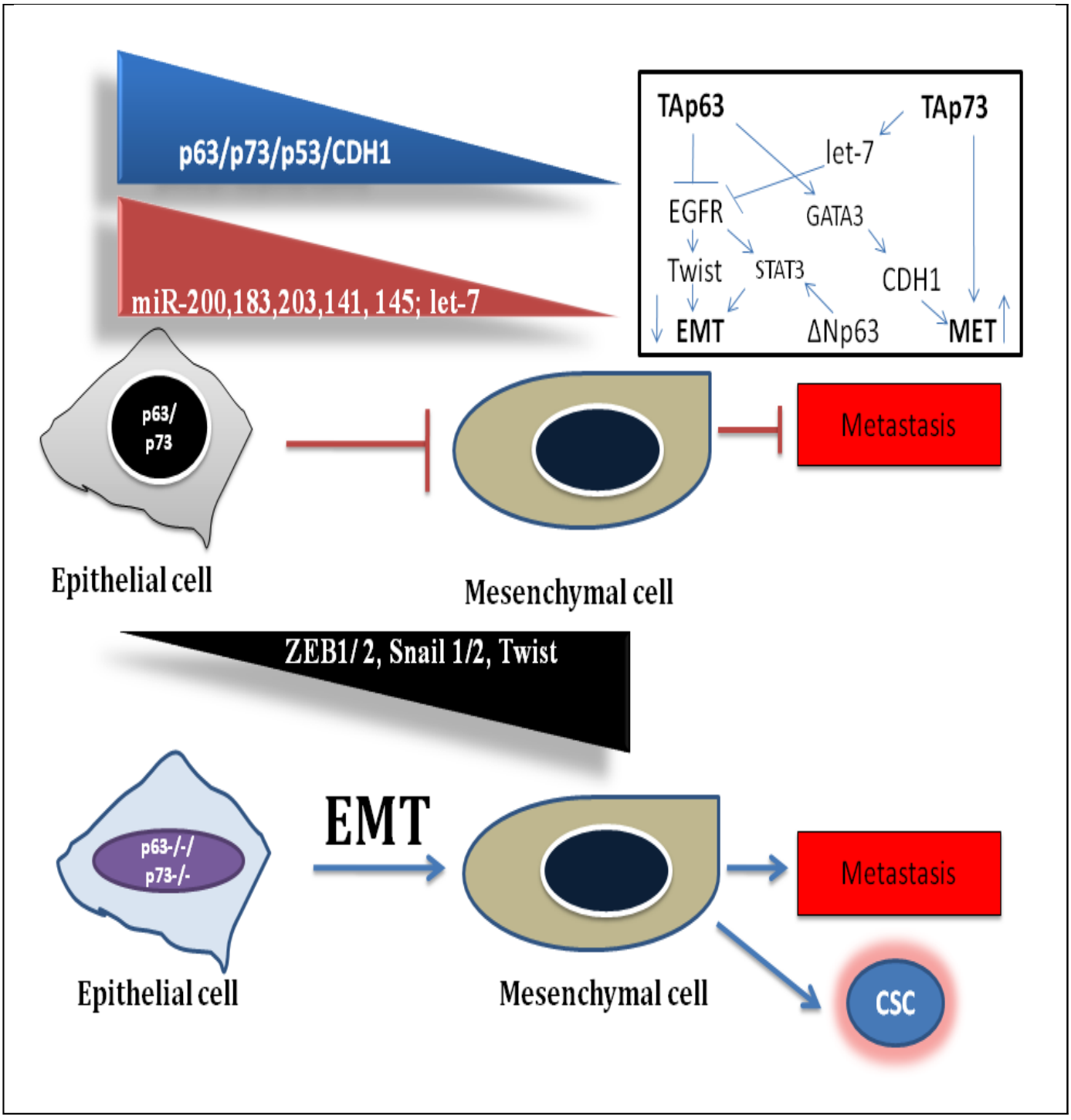


Figure 9 p53/TA-p63/p73 inhibits Epithelial to mesenchymal transition (EMT), but promotes Mesenchymal to epithelial transition (MET).

Tables

No	Position	miR-203 promoter sequence; p53/p63/p73-RE elements
1	-171 to -197	(cggctgggat)cccccag(cgccaggcga)
2	-200 to -226	(cagcgaggac)gcggcg(gggctggct)
3	-406 to -442	(gagcaggtcc)ccg(ggccgtggag)gatc(agtcgcggga)
4	-558 to -600	(gcccgagcac)ccccggccc(agacgagacg)gttc(gggcgtggcc)
5	-926 to -949	(gagcgaggct)cag(gcccttgctg)
6	-2552 to -2582	(agacaggctt)ggagc(gttcgtgtcc)tg(cgccgcgttg)
7	-4566 to-4587	(ggacgtgact)t(ggccaagtgg)

Table 1 Human miR-203 promoter contains p53/p63/p73 responsive elements

No	Position	miR-183 promoter sequence; p53/p63/p73RE elements
1	-27 to -50	(gagcaggaa)cggg(catcgtggc)
2	-52 to -73	(ccccaaggga)gt(gggcaggcta)
3	-146 to -182	(ctccttgaag)gt(catcttggc)tgatg(gggcatgtgg)
4	-357 to -376	(ctgcttgcct)(ctccgagcca)
5	-2406 to -2427	(gggcgtggca)cc(cccaggcac)
6	-3205 to -3233	(tgacaggcgc)agcgtcca(gggcatgccc*)
7	-4873 to -4590	(gggcgcgctt*)caagctcc(gtccatgcgg)

* Perfect p53-RE half-site

Table 2 Human miR-183 promoter contains p53/p63/p73 responsive elements

No	Position	miR-200b promoter sequence; p53/p63/p73RE elements
1	-348 to -367	(agacaaggca)(gcacgtgggg)
2	-376 to -400	(atcctggaga)cagag(gcccttgtcc)
3	-488 to -514	(cagcatggga)gagggg(tctccaggtgg)
4	-1130 to -1153	(ggtcaggacc)tcgg(agacgtggcc)
5	-1473 to -1494	(aggccggccc*)gt(ccgccgggtg)
6	-2636 to -2673	(aaacgagttt*)gtcagaacatttttag(tatctagagg)
7	-3504 to -3525	(ggcctggca)ga(gggcaggctc*)
8	-3871 to -3893	(aaccggctc)ctg(ggccgcggcc)
9	-4231 to -4251	(aggcgagccc*)c(cgccgagccg)
10	-4384 to -4403	(gggcctgcgg)(ggccggggcc)
11	-4519 to --4539	(aggcgagagt)t(gcccgcggct)
12	-4669 to -4696	(gtccatgaga)ggcctcag(cccatggga)
13	-4802 to -4821	(ctccgagccc)c(tggcgaggag)
14	-4852 to -4871	(ggtctcggag)(cctcttgccc)
15	-4953 to -4972	(gttccgggag)(ctgctggggc)

* Perfect p53-RE half-site

Table 3 Human miR-200b promoter contains p53/p63/p73 responsive elements

No	Position	miR-200c promoter sequence; p53/p63/p73RE elements
1	-113 to -132	(cagcagggct)(caccaggaag)
2	-578 to -598	(gcccgtggct)g(gcgctgggag)
3	-609 to -631	(ggcctagagg)agt(ggccaagcct)
4	-1164 to -1144	(atacaagccg)cagctgcacaa(aggcaagtcc*)
5	-2218 to -2241	(gcacgtggga)gggt(gaccgtgggt)
6	-3168 to -3139	(ggacaagtgt)tg(cagctgggg)
7	-3804 to -3823	(ttcccgggga)(gggcttgact)
8	-3949 to -3968	(gcccgaggtg)(gagcgtgtcc*)

* Perfect p53-RE half-site

Table 4 Human miR-200c promoter contains p53/p63/p73 responsive elements

No	Position	miR-141 promoter sequence; p53/p63/p73RE elements
1	-566 to -588	(ctgcttggac)tgc(aacctgggcc)
2	-1562 to - 1592	(atacaagccg)cagctgcacaa(aggcaagtcc*)
3	-5333 to -5352	(gagctggcct*)(ggcctgggtg)
4	-5851 to -5871	(accctgggtg)(gatcgtggct)
5	-6121 to -6140	(gagctggccc*)(gaccatgggg)
6	-6212 to -6257	(ggacttgttc*)tcctctctggtcgggtagggtgagatggatgaggtg(ttccgagaga)
7	-7592 to -7623	(agccgtgtca)t(cgtcatgacc)(acccgagagg)
8	-7820 to -7845	(cagctgggca)aagccg(aagctggctt*)
9	-8455 to -8478	(aagcaggagg)tgaa(gaacttgcac)
10	-9279 to -9299	(ctgcaggcca)a(gggcgagccc*)
11	-9582 to -9608	(aagcttgccc*)tagagtc(agtcaagggc)
12	-9662 to -9687	(tggcttgaac)ctggga(ggtcgaggct)

* Perfect p53-RE half-site

Table 5 Human miR-141 promoter contains p53/p63/p73 responsive elements

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