

Marta Diaz Martinez

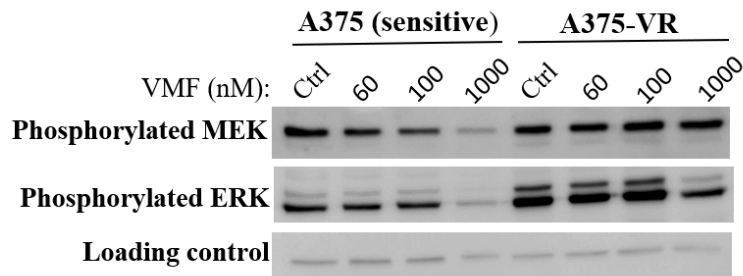
Designing Western blots, qPCR, and proliferation assay experiments to learn how cells acquire resistance to VMF.

I. Western Blot

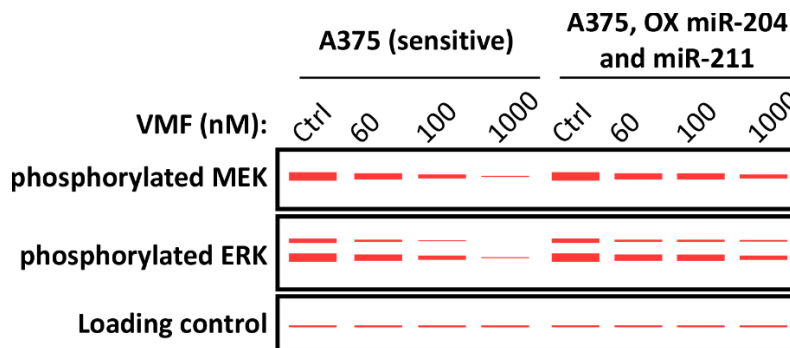
(a) Comparing A375 (VMF-sensitive) cells with A375-VR (VMF-resistant) cells, you can use a Western Blot to measure the abundance of a protein in each type of cell.

Western Blots can be designed to specifically identify phosphorylated proteins (without identifying the unphosphorylated copies of the same protein). How does the Western Bot data below indicate how cells acquire VMF resistance?

Cells acquire VMF resistance by continuing to phosphorylate MEK and ERK in the presence of VMF.



(b) To test our model, we engineer A375 cells to **overexpress** miR-204 and miR-211 and you do the same experiments as above in (a). Use the blank Western Blots below to sketch the bands you predict to see for A375 cells and these engineered A375 cells with over-expressed (“OX”) miR-204 and 211?

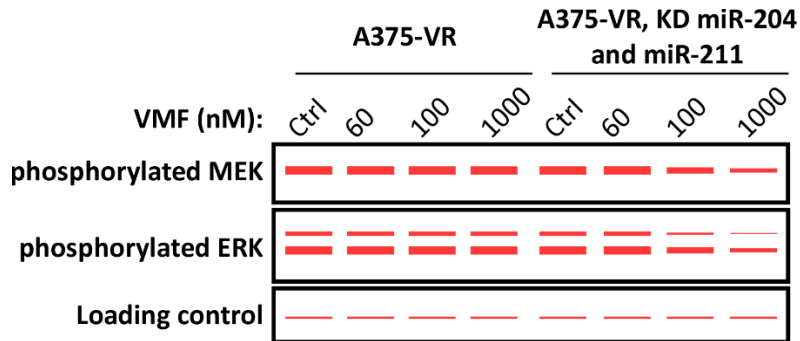


(A375, OX miR-204 and 211 cells behave more like A375-VR cells, and maintain high levels of MEK and ERK phosphorylation in the presence of VMF, but not as much as A375-VR cells.)

(c) To conduct the experiment described in part (b), would you use **microRNA mimics** or **antagomirs** to overexpress miR-204 and miR-211?

microRNA mimics

(d) To test our model, we engineer A375-VR cells to **knockdown** miR-204 and miR-211 and you do the same experiments as above in (a) and (b). Use the blank Western Blots below to sketch the bands you predict to see for the A375-VR cells and these engineered A375-VR cells with knocked-down (“KD”) miR-204 and 211?



(A375-VR, KD miR-204 and 211 cells maintain phosphorylation of MEK and ERK in the presence of VMF, but not as much as A375-VR cells.)

(e) To conduct the experiment described in part (d), would you use **microRNA mimics** or **antagomirs** to knockdown expression of miR-204 and miR-211?

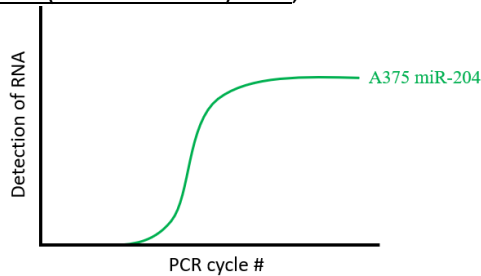
Antagomirs

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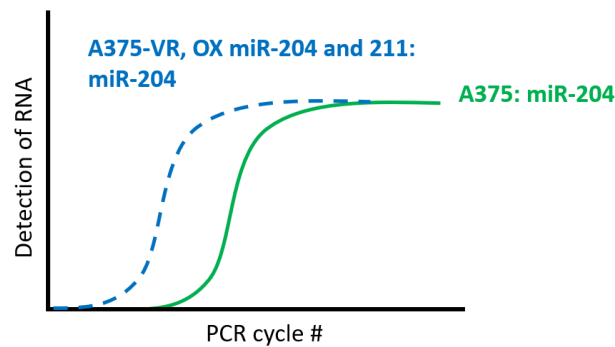
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II. qPCR Assays and Proliferation Assays

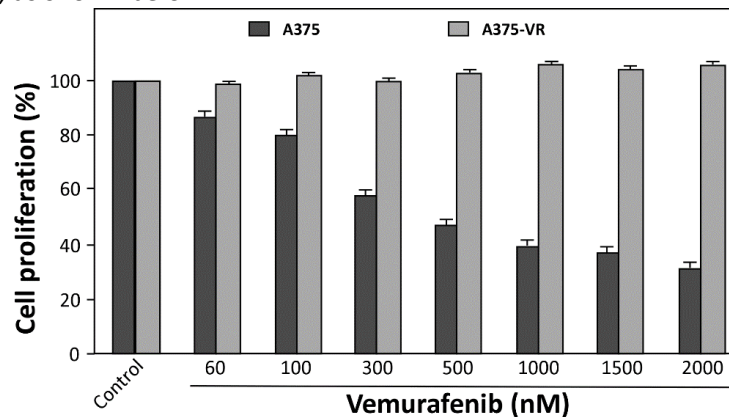
A qPCR assay is conducted on A375 (VMF-sensitive) cells, with the results shown below:



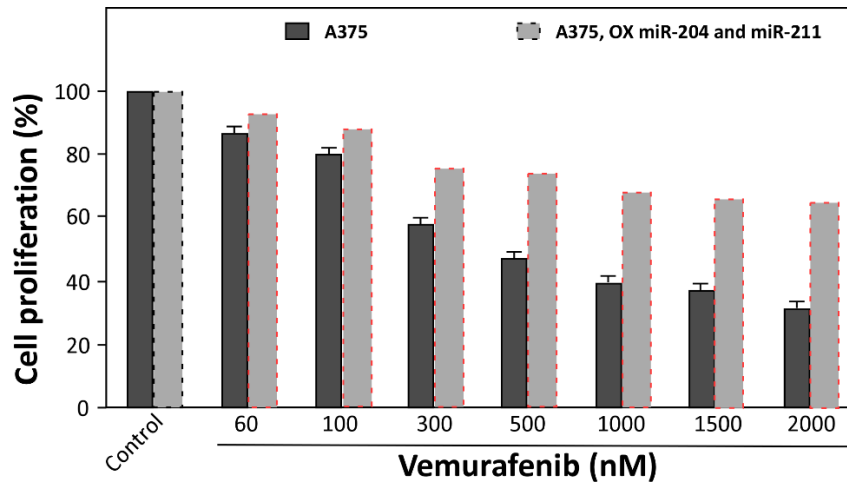
(a) To test our model, we engineer A375 cells to **overexpress** miR-204 and miR-211, and you do another qPCR of the A375 engineered cells to confirm miR-204 and miR-211 are **over-expressed**. Use the graph below to draw a dashed line where you predict to see the miR-204 data for these engineered A375 cells with over-expressed (“OX”) miR-204.



We can conduct proliferation assay experiments to compare A375 (VMF-sensitive) cells with A375-VR (VMF-resistant) cells, as shown below:



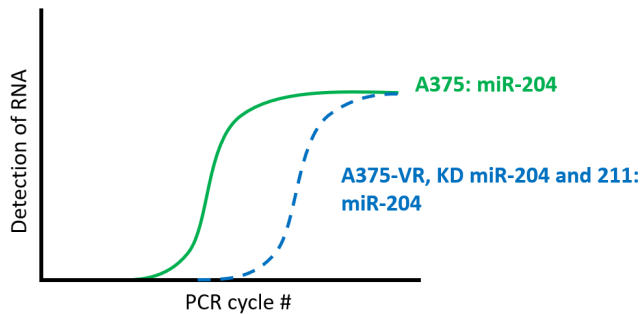
(b) To test our model, we engineer A375 cells to **over express** miR-204 and miR-211 and you do the same proliferation assay as shown above. Use the graph below to draw dashed boxes where you predict see the data from these engineered A375 cells with over-expressed (“OX”) miR-204 and miR-211:



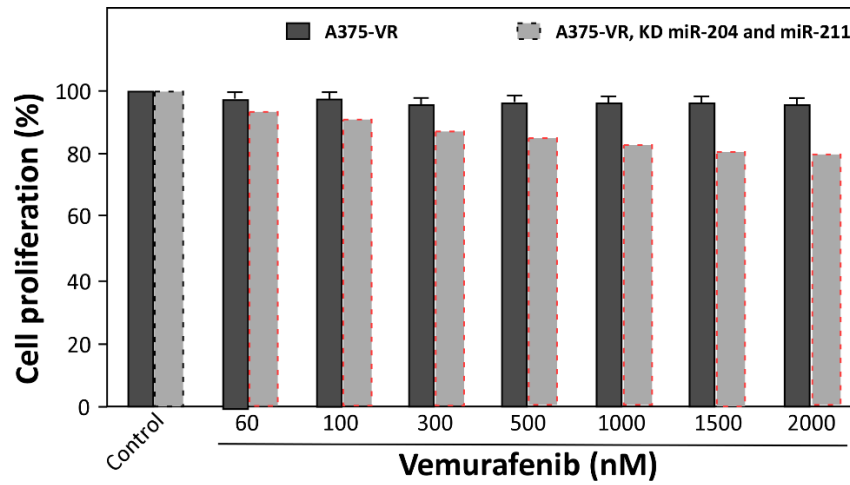
(c) To conduct the experiment described in part (b), would you use **microRNA mimics** or **antagomirs** of miR-204 and 211?

microRNA mimics

(d) To test our model, we engineer A375-VR cells to **knockdown** miR-204 and miR-211, and you do another qPCR of the A375-VR engineered cells to confirm miR-204 and miR-211 are **knocked-down**. Use the graph below to draw a dashed line where you predict to see the miR-204 data for these engineered A375-VR cells with knocked-down (“KD”) miR-204.



(e) To test our model, we engineer A375-VR cells to **knockdown** miR-204 and miR-211 and you do the same proliferation assay as shown above. Use the graph below to draw dashed boxes where you predict see the proliferation data from these engineered A375-VR cells with **knocked-down** (“KD”) miR-204 and miR-211:



(f) To conduct the experiment described in parts (d) and (e), would you use **microRNA mimics** or **antagomirs** of miR-204 and 211?

Antagomirs