**Towards decoding the coupled decision-making of epithelial-mesenchymal transition and metabolic reprogramming in cancer**

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**Abstract**

Abnormal metabolism and metastasis are two hallmarks of cancer. During metastasis, a developmental program, epithelial-mesenchymal transition (EMT) is often used by cancer cells to become motile. Aside from complete EMT, cancer cells can alternatively acquire a hybrid epithelial/mesenchymal (E/M) phenotype, which combines the features of E and M and in many cases serves as the primary instigator of metastasis. Cancer cells have been observed to largely use glycolysis irrespective of the presence of oxygen, referred to as the Warburg effect (W). In addition to glycolysis, when leaving the primary tumor and entering blood circulation, cancer cells can increase their mitochondrial oxidative phosphorylation (OXPHOS, O) without compromising their glycolytic activity, thus entering a hybrid metabolic mode (W/O). The W/O state have been often observed to be associated with enhanced metastatic potentials. Understanding the relationship between cancer metabolism and EMT can therefore offer novel anti-metastasis strategies. Here, we analyze the relationship between metabolism and EMT by coupling their corresponding core decision-making molecular networks – AMPK/HIF-1/ROS and miR-34/SNAIL/miR-200/ZEB, respectively. We systematically elucidate how different phenotypes during EMT (E, M and hybrid E/M) are associated with different metabolic phenotypes (O, W and hybrid W/O). Specifically, we identified the feedback loops that lead to the coupling of the E/M state with the W/O state, referred to as E/M-W/O, – a potentially highly aggressive phenotype. Strikingly, we found that even if the individual molecular network of EMT or metabolism does not support a hybrid phenotype, the crosstalk with the other can give rise to the E/M-W/O state. Moreover, in this case, there is an order of events in that W/O state emerges first and followed by the E/M state, suggesting that metabolic reprogramming can be a primary driver of EMT and the acquisition of highly metastatic hybrid E/M cells.

**Introduction**

Metastasis remains the leading cause of cancer-related deaths[1] and thus it is critical to understand the physiological properties of cells that migrate from the primary tumor and initiate metastatic lesions. Typically, these properties have been studied one at a time. For example, cell motility is assumed to be related to the epithelial-mesenchymal transition (EMT). During EMT, the cells progressively lose epithelial (E) features such as cell-cell adhesion and apical-basal polarity, and acquire mesenchymal (M) features such as migration, invasion, and resistance to immune response [2,3]. The EMT has consistently been implicated in cells acquiring metastatic potential [4,5], and also plays a role in therapeutic resistance [6]. Recently, the bimodal picture of EMT has been superseded by a more complex scenario involving the hybrid epithelial/mesenchymal (E/M) phenotype which exhibits combined traits of epithelial (cell-cell adhesion) and mesenchymal (invasion) at the single-cell level. The hybrid E/M cells migrate collectively as a cluster and may account for the majority of metastases [7–10]. The existence of a hybrid E/M state has since been experimentally verified both in vitro (in many cancer cell lines) and in vivo (e.g. using a genetic mouse model of squamous cell carcinoma) and has been shown to be associated with therapy resistance alongside with poor survival rates [11–14]. Most importantly, these states appear to the most capable of initiating metastatic growth[15,16]. Fully understanding the behavior of the hybrid E/M phenotype is still an active area of research.

Metabolic reprogramming, another hallmark of cancer, enables cancer cells to adjust their metabolic activity for biomass and energy supply to survive in hostile environments [1,17].Normal cells typically utilize oxidative phosphorylation (OXPHOS, O) under normoxic conditions and glycolysis when there is a lack of oxygen. However, cancer cells often prefer glycolysis even when oxygen is available, referred to as the Warburg effect (W) or aerobic glycolysis [18,19]. During metastasis, cancer cells must be able to adjust their metabolic phenotype in order to survive in varying environments, resulting in these cells switching between different types of metabolism [20–23]. Metabolic reprogramming, specifically in the context of switching between the O state and W state, can enable cancer cells mix and match different metabolic models, leading to the acquisition of a hybrid W/O phenotype and a metabolic low-low phenotype (L/L). The W/O cells actively use both glycolysis and OXPHOS and are often associated with enhanced metabolic potentials [24–26] [24,28]. The L/L cells are metabolically inactive exhibiting both low glycolysis and low oxphos, and being associated with therapy resistance in melanoma [27]. The highly metastatic murine breast cancer 4T1 cells exhibit both higher glycolytic and oxphos activity relative to the isogenic and less metastatic 67NR cells (ref. F Dupuy, et al., PDK1-dependent metabolic reprogramming dictates metastatic potential in breast cancer. Cell Metab 22, 577–589 (2015).). Furthermore, when the circulating tumor cells (CTCs) formed by 4T-1 cells exhibited enhanced oxphos relative to both the primary tumor and lung metastasis formed by 4T1 [29]. The high metastatic potential enabled by the hybrid metabolic phenotype has been confirmed in a number of additional experimental studies [28,30]. Together, these experiments suggest a tight connection between metabolic plasticity and cancer metastasis, specifically the hybrid W/O state with high metastatic potential.

As already mentioned, many studies mostly focused on either only EMT or only metabolism (Madeline, please add appropriate references here). However, it has become increasingly clear that there exists extensive crosstalk between EMT and metabolism[30]. (Please briefly mention 2-3 examples showing how EMT factors can regulatie metabolism and vice versa). Understanding the crosstalk between EMT and metabolic reprogramming is important to better understand metastasis and tumor proliferation [30–33]. Recent studies show that metabolic reprogramming can increase metastatic potential and drive EMT, or conversely that induction of EMT can drive metabolic reprogramming [34–37]. The underlying mechanisms of interaction between EMT and metabolic reprogramming remain poorly understood, with several competing hypotheses as discussed below. Kang et al suggested cancer cells typically undergo metabolic reprogramming first and then trigger EMT [38,39]; this coupling, presumably, is a consequence of changes in the tumor microenvironment fostering metabolic reprogramming which drives EMT[40–42]. Another hypothesis is that the mutual activation between EMT and metabolic reprogramming can contribute to flexible coupling of various EMT states (E, M, E/M) with different metabolism states (W, O, W/O) and possibly the two hybrid phenotypes (E/M and W/O)) become coupled under certain cross-talk, leading to a greatly increased metastatic potential[30]. One supporting evidence has recently been noticed in CTCs, where the CTCs exhibit enhanced oxphos and no compromise in glycolysis[29] and have also been shown to mainly consist of hybrid E/M cells, especially at high levels of NRF2, an antioxidation regulator[43]. Consistent coupling of E/M and W/O has been seen in breast cancer stem cells (BCSCs). Specifically, the hybrid E/M-like BCSCs (E/M-BCSCs) exhibit higher levels of both OXPHOS and glycolysis as compared to the mesenchymal-like BCSCs (M-BCSCs) [44,45]. While there have been preliminary indications of the coupling of EMT states and metabolic states, a systematical analysis of how different EMT and metabolism states are coupled remain to explored.

To decode the coupled decision-making of EMT and metabolism, we developed a mathematical model which couples the core gene regulatory circuit of EMT – miR-34/SNAIL/miR-200/ZEB [7] with that metabolism – AMPK/HIF-1/ROS [25]. By analyzing the coupled circuit, we found that ROS is a key promoter of “double-hybrid” state, namely the hybrid E/M state coupled with the hybrid metabolic phenotype, referred to as the E/M-W/O state. Additionally, HIF-1 may play a more central role in metabolic reprogramming driving EMT than AMPK. Strikingly, we found that there are parameter space regions for which the E/M-W/O state is the only accessible state with bi-directional crosstalk. Interestingly, even if the individual circuit can’t give rise to the hybrid phenotype, (i.e., neither the E/M or W/O states are initially accessible), once including crosstalk, the hybrid states (E/M or W/O) emerge. Indeed, a single crosstalk is sufficient for the metabolism or EMT circuit to gain tristability. We also show that our previously identified phenotypic stability factors (PSFs) of the E/M state - GRHL2 and OVOL2 [14,46], ont only stabilize E/M but also stabilize the E/M-W/O state. Our results therefore suggest that a highly aggressive plastic phenotype along both the EMT and metabolic axes ()E/M-W/O is a likely choice for a subset of cancer cells and, speculatively, may be critical for metastases.

**Model: Coupling the regulatory networks of EMT and metabolism**

While the mechanisms of EMT and cancer metabolism have been investigated individually, the crosstalk between the two circuits and how the phenotypes are correlated is still largely unknown. To decode the cross-talk between EMT and metabolism, we couple our previously published regulatory networks of EMT [7] and metabolism [25] by including the mutual regulatory links between these two circuits; see Figure 1A for the coupled network and see SI for details of each of the crosstalk.. The crosstalk between the EMT circuit and the metabolism circuit can be direct (e.g., ) or indirect (e.g., ), the latter arising because our formulation focuses only on a few core components and effective interactions between them that can occur via intermediate reactants. We initially focus on the core networks and investigate the role of crosstalk on the coupling of different EMT and metabolism states. Then we ask an interesting question - whether the crosstalk contribute to the mergence of the hybrid states (E/M, W/O). Lastly, we evaluate the role of thePSFs of E/M, OVOL and GRHL2 on the stability of the E/M-W/O state.

The core EMT network is comprised of the EMT-inducing transcription factors (EMT-TFs), ZEB and SNAIL, and the microRNA families, μ200 and μ34. It is modeled as a transcription-translation chimeric circuit[7]. For a two-component chimeric circuit consisting of one microRNA (u) and one TF (RNA, m, protein, B), the binding/unbinding dynamics are given by

(1)

(2)

(3)

where the three functions, , and which represent respectively the active miRNA degradation rate, active mRNA degradation rate, and translation rate (details in SI section 1.1, Fig. S1-S3). The transcriptional activation and inhibition by SNAIL and ZEB are mathematically represented as a shifted Hill function[47] which is defined as shown below,

(4)

The fold change () represents the magnitude of the activation ( >1) or inhibition (0=< <1), and the sensitivity to the changes in X is represented by the Hill coefficient n (Fig. S3). Previous investigation of the core EMT network by Lu and collaborators showed that the miR-200/ZEB module was responsible for the EMT tristability - epithelial (E) with high miR-200/low ZEB, mesenchymal (M) with low miR-200/high ZEB, and E/M with intermediate miR-200/ intermediate ZEB , whereas the miR-34/SNAIL module mainly acted as a noise buffer[7](see Fig. 1B, and section S2.1 for nullcline analysis).

In a separate line of investigation, a proposed generic regulatory circuit of metabolism - AMPK/HIF-1/ROS, provided insight into cancer metabolism plasticity and switching between different metabolism phenotypes. Through this reduced circuit, Yu and collaborators show that cancer cells can acquire at least three different metabolic phenotypes – an “O” state (high AMPK/low HIF-1), a ‘W’ state (low AMPK/high HIF-1) and a hybrid ‘W/O’ state ()1 (see Fig. 1C).

To couple the regulatory circuits of EMT and metabolism, we did extensive literature search and identified main crosstalk between these two circuits (see Fig. 1A). The crosstalk is obviously bi-directional. For example, regarding EMT regulating metabolism, can up-regulate ROS via downregulating the NRF2-dependent antioxidant capability, [48–50] or via . This increase in ROS levels by is potentially more pronounced for mitochondrial ROS (mtROS) versus NADPH oxidase mediated ROS (noxROS) [48]. Next, family members can either upregulate or downregulate Hif1 expression [55]. While miR-429 upregulates HIF-1, both miR-200b [56] and miR-200c [57] downregulate HIF-1 expression. (Please add one sentence to say why we focused on the overall negative regulation of HIF-1 by miR-200) Regarding metabolism regulating EMT, HIF-1 inhibits miR-200b through upregulation of HIF-1 downstream target ASCL2 [56]. Therefore, there is a mutual inhibitory feedback loop between and HIF-1. Additionally, HIF-1 can directly? upregulate SNAIL [58], while AMPK represses the production of SNAIL[59] by activating FOXO3. Similarly, AMPK suppresses ZEB by activating FOXO [60,61]. Additionally, CREB, after being activated by AMPK via phosphorylation, can transcribe resulting in the upregulation of [62–66]. Please refer to supplementary Table S5 for a detailed description of all crosstalk that have been included in our modeling framework.

The new model we propose here is built by including these crosstalk links so as to couple the circuits of EMT and metabolism respectively. The full equations for the dynamics of all components of the circuit are given in SI Section 1.3 and the parameters along with a brief explanation are given in SI Section 1.4.

We started with parameters such that both the EMT and metabolic networks are tristable. This means when the crosstalk are inactive, there are maximum nine possible combinations of the EMT and metabolic phenotypes: E-W, E-O, E-W/O, M-W, M-O, M-W/O, E/M-W, E/M-O, and E/M-W/O (Fig. 1D, details of numerical integration using the Euler method are given in section S2.2). By including active crosstalk, we can identify how the crosstalk affects the coupling between EMT states and metabolism states.

While the W state is characterized by high HIF-1/low AMPK and the E state is characterized by high /low ZEB expression, including the crosstalk will quantitatively alter the expression profiles for the various steady states. This means that the use of fixed thresholds to determine the state of the cell is no longer appropriate. Therefore, we use a distance metric normalized by the expression of the decoupled network to classify the generated expression profiles as indicative of one of the nine coupled states (see Section S2.3 for details). With our baseline decoupled network parameters, we show that 1000 initial conditions are large enough to generate consistent percentages of different states (Fig. S5-S7) - with the hybrid states being most populous (W/O and E/M) followed by the W and M phenotypes, followed by the O and E states. This result is just for one set of parameters and others will lead to a different fraction of initial conditions leading to these disparate states.



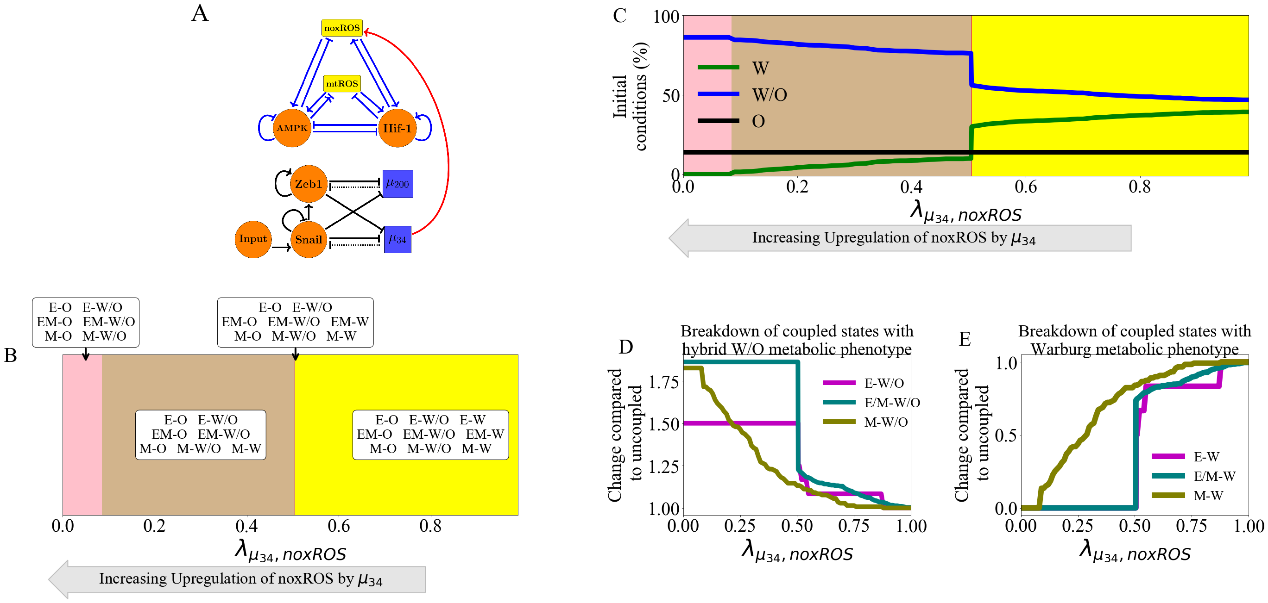
**Figure 1**. **The coupled EMT/MR circuit results in 9 possible steady states.** With inactive crosstalk all combinations of the steady states of the core EMT and metabolic networks are accessible. **(A)** The network showing the core EMT module (bottom) with regulatory links designated by black, the core metabolic circuit (top) with regulatory links designated by blue, and the crosstalk noted in red. The dashed lines denote miRNA-based regulation. The solid lines denote transcriptional regulation. Regulatory links ending in bars represent inhibition while the arrows represent activation. **(B)** The nullclines of the EMT network. The system is tristable and the three stable states (high /low Zeb, low /high ZEB, and intermediate /ZEB) represent the three phenotypes (E, M, E/M) respectively. **(C)** The nullclines of the metabolic network. The system is tristable with three stable states (high AMPK/low HIF-1, low AMPK/high HIF-1, and intermediate AMPK/HIF-1) represent the three metabolic phenotypes (O, W, W/O) respectively. **(D)** The 9 possible phenotypic states when all crosstalk are inactive. The blue, purple, and black markers represent the M, E/M, and E states, respectively. The circle, cross, and square represent the Warburg W, W/O, and O states, respectively. Therefore, the E/M-W/O state is represented as a purple cross.

**Results**

**Individual crosstalk can push the downstream circuit towards a single state**

Let us start by making just one cross-link active, caused by e.g. an EMT-related microRNA. Now, in our model there is a clearly an unaffected upstream subnetwork (EMT, from where the link originates) and a regulated downstream one. (Note that the model ignores any possible dilution of the microRNA due to its action on ROS; see below).

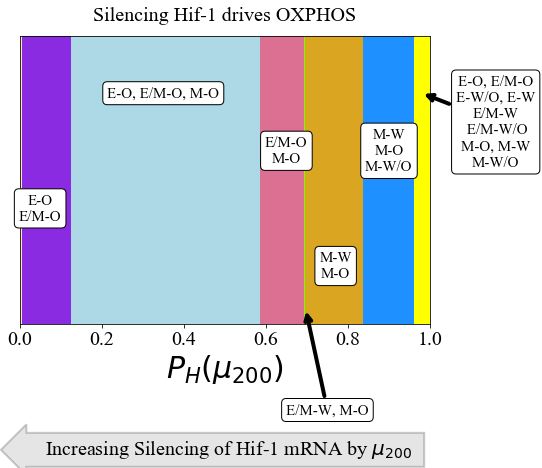
Analyzing the states coupled with the W state, we found that the coupled M-W state persists longer as the coupling is increased, as expected since it has the lowest level (Fig. 2E). Similar changes have been observed via the upregulation of mtROS (Fig. S8); the E-W and E/M-W states are also the first suppressed states. Consistent with the effect of upregulating noxROS, upregulating mtROS is also correlated with an increase of the E/M-W/O state. Further, activation of mtROS results in a downregulation of the O state alongside downregulation of the W state, thus stablizin g the W/O state. Together, these results suggest ROS, including both mtROS and noxROS, is a critical factor in regualting the coupling of two hybrid states - W/O E/M, and mtROS exhibited a more pronounced effect than noxROS.



**Figure 2. noxROS upregulated by miR-34 stablizes the W/O state and enhances the E/M-W/O coupled state.** As noxROS is upregulated by miR-34, the number of initial conditions leading to the O state is minimally changed, the W state is reduced, and the W/O state is increased. The EMT network is unchanged due to no feedback but the coupling of metabolic states with the EMT states changes,. **(A)** A diagram of the core EMT circuit (left) and the core metabolic circuit (right) connected by the crosstalk - upregulating noxROS (red link representing transcriptional regulation). **(B)** Of the nine possible coupled states, as noxROS is upregulated by miR-34, there are 4 distinct groupings. All possible couplings of the EMT phenotypes (E, M, and E/M) with both the O and W/O metabolic phenotypes persist for all levels of noxROS upregulation. The coupled states associated with the W metabolic phenotypes, (E-W, E/M-W, and M-W), are lost as the level of noxROS increases, as shown in the red, tan, and pink regions, respectively. **(C)** The W/O phenotype (blue) is upregulated, Warburg (green) phenotype is downregulated, and OXPHOS (black) is unchanged. The lines represent the total number of initial conditions leading to the W, O, or W/O phenotypes as a function of increasing regulation of noxROS by miR-34. **(D)** Showing the breakdown of the coupled states associated with the W/O phenotype (i.e., E-W/O, M-W/O, and E/M-W/O) compared to the inactive system (). The E/M-W/O coupled state is greatly upregulated once , the M-W/O coupled state is slowly upregulated, and E-W/O is also upregulated. **(E)** Same as (D) but for the coupled states associated with the Warburg phenotype. Once , both the E-W and M-W states are fully suppressed. The E/M-W coupled state continues to be downregulated until it is fully suppressed near .

**Regulation of HIF-1 affects both subcircuits:**

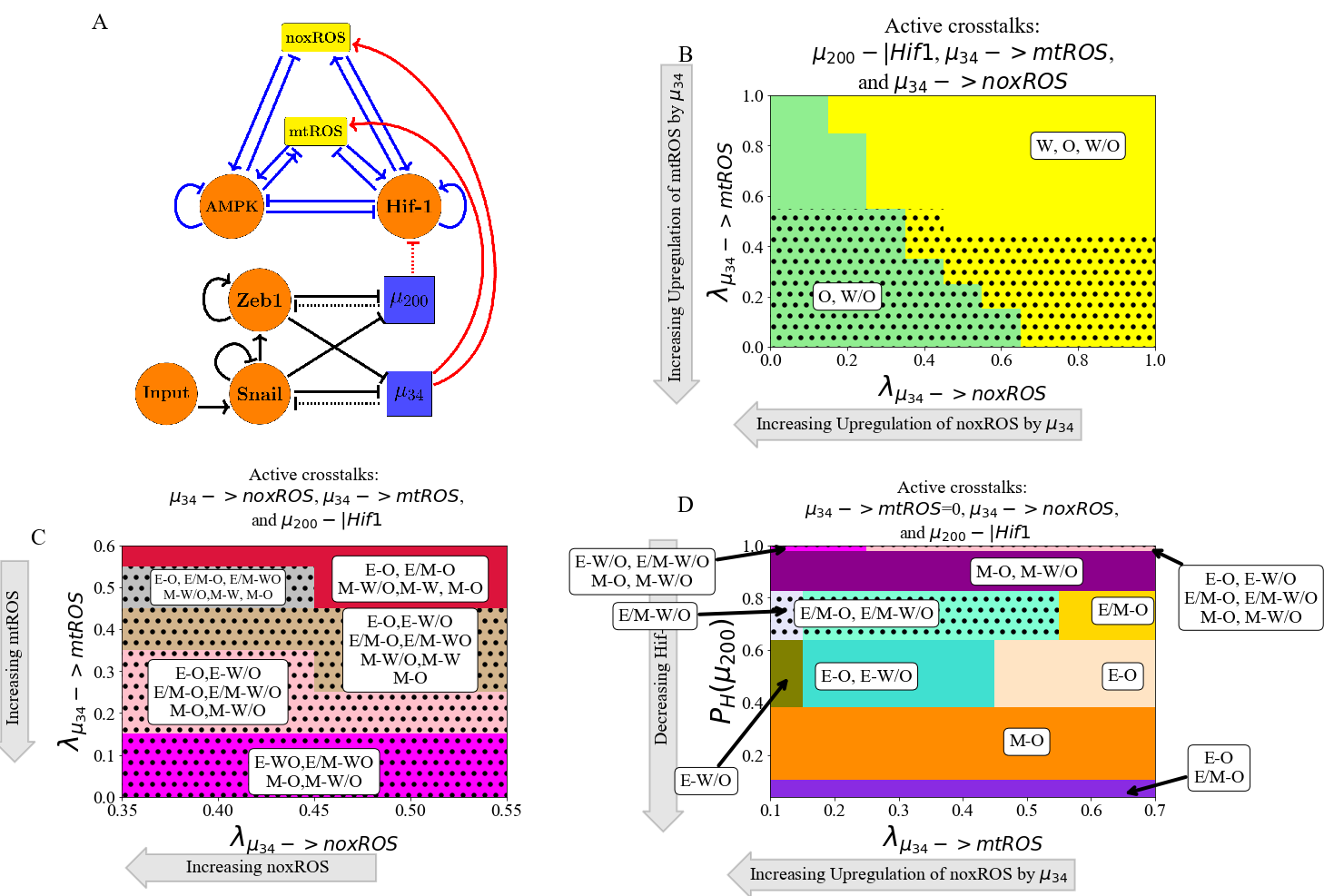
While the previous link only affected the downstream network, the miRNA regulation of HIF-1 by can affect both networks. This arises because of the reduction in the microRNA level caused by this coupling. In our model, miR-200 mediates both the transcription and translation of HIF-1 mRNA, and as a result, miR-200 can be recycled or degraded. Therefore, while the downstream metabolic network is modulated, the upstream EMT network is also affected via change of We have defined a function to simulate the above-mentioned effect of miR-200 on HIF-1 (detail of silencing function in section S2.5). Note that as we include increased silencing, the first thing which occurs is the restriction of the EMT state; close to the only EMT state allowed is M. When we enter this region, all the metabolic phenotypes are allowed. As the miR-200 mediated regualtion of HIF-1 increases, the W/O and W states are suppressed sequentially and the O state promoted. As the HIF-1 is suppressed by miR-200, the degradation of due to binding to HIF-1 RNA is reduced and as a result and the W state gradually disappears. When HIF-1 mRNA is fully silenced, only the E-O and E/M-O coupled states remain (Fig. 3). Since the E/M state does not reappear until after the metabolic system has fully transitioned to O, the coupled E/M-W/O state is not observed for all values of silencing HIF-1 mRNA. These results suggest overexpression could promotes the O-associated states (O-E and O-E/M) and destabilize the coupled E/M-W/O.

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**Figure 3.** **The coupled phenotypes associated with increased silencing of the HIF-1 mRNA by .** Both the EMT and metabolic networks are affected by the miR-200-mediated regulation of HIF-1. We started with the EMT network with M as the only stable state. At minimal silencing (PH( near 1) only the coupled states with M are accessible (M-W, M-O, and M-W/O). Then as miR-200-mediated inhibition of HIF-1 increases the W/O state is lost, then the M-W state becomes E/M-W, and after that only the coupled states with the “O” state are accessible. At complete silencing of the HIF-1 mRNA only the E-O and E/M-O states are accessible.

**Inclusion of all miRNAs of the EMT network can stabilize the W/O metabolic phenotype**: We next wish to determine how including links emanating from both miR-200 and miR-34 can synergistically drive metabolic reprogramming, and specifically enhance the chances of being in the coupled E/M-W/O state. As mentioned previously, upregulated ROS leads to an increased W/O phenotype (Fig. S8). If both noxROS and mtROS are upregulated by the E/M-W/O state is further upregulated (Fig. S15). While upregulation of ROS causes an increase in the E/M-W/O state, we showed above that silencing HIF-1 mRNA suppresses the coupled E/M-W/O state; therefore, we may expect some suppression of the E/M-W/O state when including both  and . Interestingly, the hybrid E/M-W/O state can be fully suppressed when downregulates HIF-1 and upregulates noxROS, but only partially suppressed when downregulates HIF-1 and upregulates mtROS (Fig. S16). These results suggest the type of ROS present can have different affects on the E/M-W/O state.

The coupled hybrid E/M-W/O state is stabilized if mtROS is upregulated, but noxROS upregulation has minimal effect on the E/M-W/O state. Strikingly, if all three miRNA crosstalk are active ( silencing HIF-1 mRNA and upregulating noxROS and mtROS, Fig. 4A) the E/M-W/O coupled state can be suppressed even if the W/O state is present (Fig. 4B). Further, the E/M-W/O state is present for all values of noxROS upregulation but is only present at high values of mtROS upregulation (Fig. 4B). Analyzing the coupled state phases shows the E/M-W/O state is suppressed when mtROS is only slightly upregulated (Fig. 4C). Further, the E and E/M states are associated with the O state when mtROS levels are slightly upregulated. Interestingly, the M state is coupled with O and W/O states while the E and E/M states are only coupled to the W/O phenotype when mtROS is fully upregulated . Additionally, depending on the initial conditions, if noxROS is maximally upregulated, mtROS is upregulated, and HIF-1 is partially silenced by the system can access the hybrid E/M-W/O state (Fig 4D). Also, while mtROS must be upregulated for the system to access the coupled E/M-W/O state, the E/M-W/O state is accessible for all levels of noxROS upregulation (SI Fig S17). The difference in the effect of noxROS and mtROS seems to result from the frustrated regulation of mtROS by HIF-1 and . Therefore, feedback loops between mtROS, HIF-1, , and together control the appearance of the E/M-W/O state.



**Figure 4.** **miR-200, miR-34 can upregulate the W/O phenotype.** When all three crosstalks from the EMT network (-|HIF-1, -> mtROS, and ->noxROS) are active the E/M-W/O state can be upregulated. The E/M-W/O state is accessible when mtROS is high and at intermediate silencing of HIF-1. The level of noxROS seems to have minimal effect. This suggests the //HIF-1/mtROS axis plays a significant role in stabilizing the hybrid E/M-W/O state. **(A)** Schematical illustration of the coupled metabolic (top) and EMT (bottom) regulatory network with all EMT driven regulatory links active ( upregulating mtROS, upregulating noxROS, and silencing HIF-1). **(B)** The phase plane corresponding to all EMT driven regulatory links (network pictured in A). The regulation of HIF-1 by in this phase plane corresponds to the rightmost, blue region of Fig. 3 where all metabolic phenotypes are possible. As noxROS is upregulated (right to left), the Warburg metabolic phenotype is suppressed. However, as the level of mtROS increases (top to bottom), the black dotted region appears showing the existence of the E/M-W/O coupled state, suggesting mtROS may have a stronger effect on the E/M-W/O phenotype than noxROS. **(C)** The coupled states when only EMT driven crosstalks are active ( downregulating HIF-1 and upregulating mtROS and noxROS). The E/M-W/O state exists when mtROS is upregulated. **(D)** At maximum upregulation of noxROS (=0), as mtROS increases (x-axis) and HIF-1 is silenced moderately(y-axis) there are regions where the E/M-W/O state is possible (black dotted regions).

**Metabolic reprogramming can drive EMT:**

We next turn to a consideration of information flowing in the other direction, from metabolism to EMT. To elucidate the way in which metabolic reprogramming can drive EMT, we determined the effect of each metabolism-driven crosstalk on the coupled states. First, we analyzed the links in which HIF-1 upregulates SNAIL (Fig. 5A and S9) or inhibits (Fig. S10). As expected, both HIF-1 meditated links push the system towards the M state. Further, both the E and hybrid E/M states are most associated with the O state (when HIF-1 level is realtively low) while the M state is initially associated with the W state. The correlation between the E-O and M-W states is assumed in much of the literature[30]. Similarly, modulating the EMT-inducing signals such as TGF-b that can activate SNAIL can alter the stability of the E/M state and therefore the coupled states (see Fig. S11). Opposite to the HIF-1 results, AMPK upregulating  pushes the EMT network to adopt an E state and suppresses the E/M state followed by the suppression of the M state (Fig. 5B and S12). Similarly, AMPK mediated downregulation of ZEB and/or SNAIL has a corresponding effect on the expression of the E/M state and the systems saturates near fully mesenchymal (Fig. S13 and S14). Additionally, when AMPK regualtes the EMT circuit alone, the E and M states are still most associated with the O and W states, respectively. However, the E/M state is associated with the W state. This is in direct contract to HIF-1 driven crosstalk in which the E/M state is coupled with the O state. The results suggest that neither OXPHOS nor Warburg metabolism is automatically associated with the E/M phenotype and that this state has metabolic plasticity to mix and match different metabolic phenotypes.

The crosstalk between the EMT network and metabolism network play various roles in mediating the coupled states. The miR-34 mediated upregulation of mtROS or noxROS exhibit consistent suppression of the E-W and E/M-W coupled states and stablize the E/M-W/O state. The phases of coupled states are also very similar when HIF-1 inhibits or upregulates Snail. However, the E/M-W/O state does persist longer when HIF-1 inhibits (Fig. S9 and S10). Lastly, the AMPK driven crosstalks (inhibiting ZEB or SNAIL or upregulating miR-200) are initially very similar. In fact, the phases are nearly identical when AMPK inhibits ZEB or SNAIL (Fig. S13 and S14). However, AMPK upregulating has slightly different effect on the coupled states before saturating at epithelial (Fig. S12). These similarities between the effect of different crosstalk suggests a degree of consistency of how EMT drives metabolic reprogramming, and vice versa.

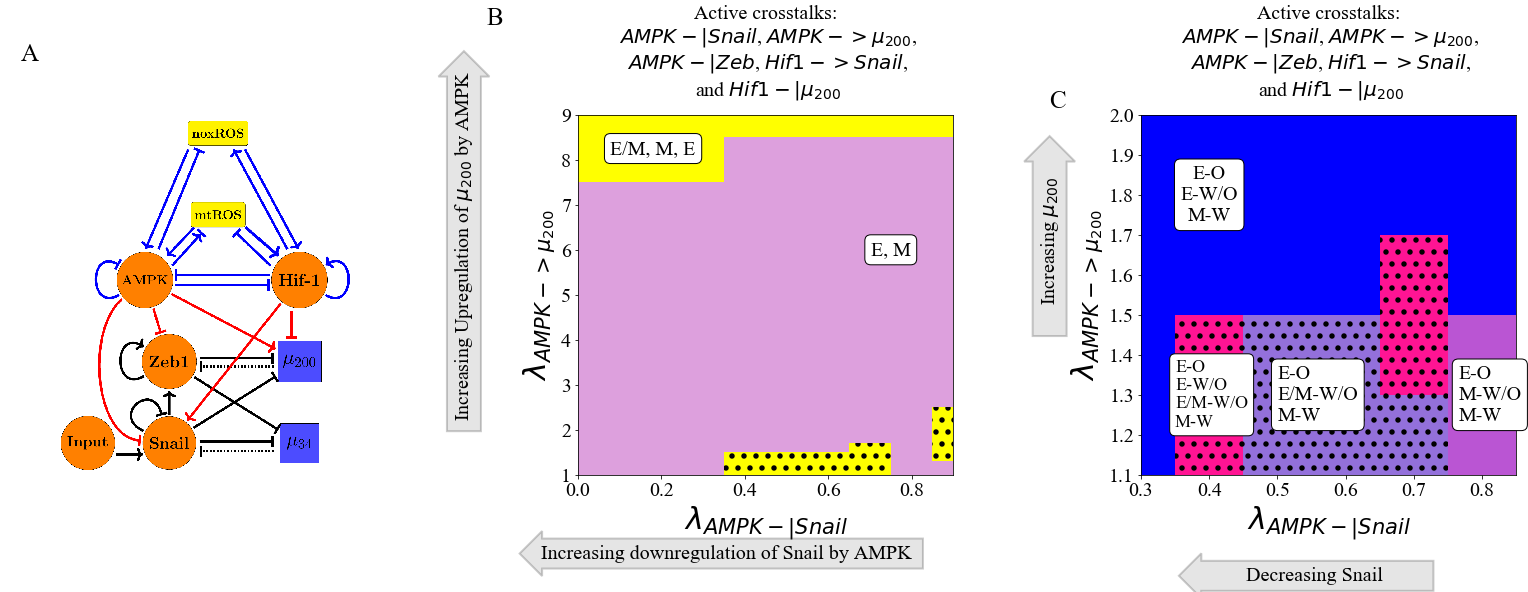
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**Figure 5. The role of metabolism in driving EMT.** HIF-1 mediated crosstalks drive the EMT circuit towards the M state, while AMPK mediated crosstalks drive the EMT network towards the E state. Neither type of crosstalk alone can stabilize the E/M state, but the E/M state persists longer for HIF-1 controlled crosstalks. **(A)** The number of initial conditions leading to an E/M, M, or E phenotype as HIF-1 upregulates SNAIL. The hybrid E/M phenotype is suppressed quickly as the system is driven towards M. **(B)** The number of initial conditions leading to an E/M, M, or E phenotype as AMPK upregulates . The E/M state persists longer for HIF-1 regulation than for that based on AMPK.

**TFs of the metabolic network can stabilize the E/M metabolic phenotype**:

There are two distinct events at play when the metabolic network regulates the EMT circuit. AMPK regulation quickly suppresses the E/M phenotype and pushes the system towards the E state whereas HIF-1 regulation can allow the system to maintain the E/M phenotype for a range of strengths while ultimately pushing the system towards M (Fig. 5A and 5B). As AMPK and HIF-1 push the system towards opposite states, having active links emanating from both should push the circuit towards a hybrid state. That is exactly what happens for any combination of the three AMPK crosstalks and two HIF-1 crosstalks, although the exact values of where the E/M-W/O state exists depends in detail on the type of regulation (Fig. S18). Additionally, if AMPK and HIF-1 target different EMT TFs, the E/M-W/O state may exist in large parameter space than if they target the same EMT TF (Fig. S18), suggesting multiple crosstalks should be active and multiple gene regulators should be targeted to stabilize the E/M-W/O state. If all crosstalks involving AMPK and HIF-1 regulating the EMT circuit are active (Fig. 6A) then there are significant regions in which the E/M state exists (Fig. 6B). However, when analyzing the system for the existence of the E/M-W/O phenotype, it only exists in a small region where is minimally upregulated. Moreover, HIF-1 driven crosstalks are able to maintain the E/M phenotype longer than AMPK driven crosstalks suggesting, the reduced regions of E/M-W/O existence is likely due to the suppression of the E/M state by AMPK regulated crosstalks, as mentioned above (see Fig. S12-S14). This suggests HIF-1 driven crosstalk may be more pronounced when considering the coupling of two hyrbdi states – E/M-W/O

To stabilize the E/M state, both AMPK and HIF-1 crosstalk are necessary, and if all EMT regulating crosstalks are active then there are regions where the E/M-W/O state exists. Additionally, the epithelial state is typically coupled to OXPHOS metabolism (E-O), the mesenchymal state is associated with the Warburg metabolic phenotype (M-W), and when the E/M state is present it is typically associated with the hybrid W/O metabolic phenotype (Fig. 6C). In fact, for any system, if there are only three coupled states available and each has a distinct phenotype of the EMT and metabolic networks, then the only possible set of states is E-O, M-W, and E/M-W/O. This behavior represents the full coordination of EMT and metabolism and suggests that cells in the primary tumor prefer to utilize OXPHOS while clusters of migrating cells utilize a combination of aerobic glycolysis and OXPHOS.



**Figure 6. AMPK and HIF-1 cooperate to upregulate the hybrid E/M state.** When all HIF-1 and AMPK controlled crosstalks are active (HIF1->Snail, HIF1-|, AMPK-|Snail, AMPK-|Zeb, AMPK-> ) the E/M-W/O state can be stabilized. HIF-1 driving the network to mesenchymal and AMPK driving the system towards the epithelial state results in HIF-1 and AMPK cooperatively stabilizing the E/M state. Once stabilized, the E/M state is coupled with the W/O state (i.e., stabilizing the coupled E/M-W/O state). **(A)** Schematical illustration of the network showing how metabolism drives EMT. ZEB is inhibited by AMPK, SNAIL is upregulated by HIF-1 while being downregulated by AMPK, and is upregulated by AMPK while being inhibited by HIF-1. **(B)** The phases plane of potential EMT phenotypes when all metabolic driven crosstalks are active. The E/M phenotype is only accessible when λAMPK-| μ200 is near 1 or very high (i.e., at the extremes of regulation). **(C)** The coupled states when the EMT circuit is regulated by the metabolic circuit (AMPK-|SNAIL, AMPK-|ZEB, AMPK->, HIF-1 -|, HIF-1 -> SNAIL).

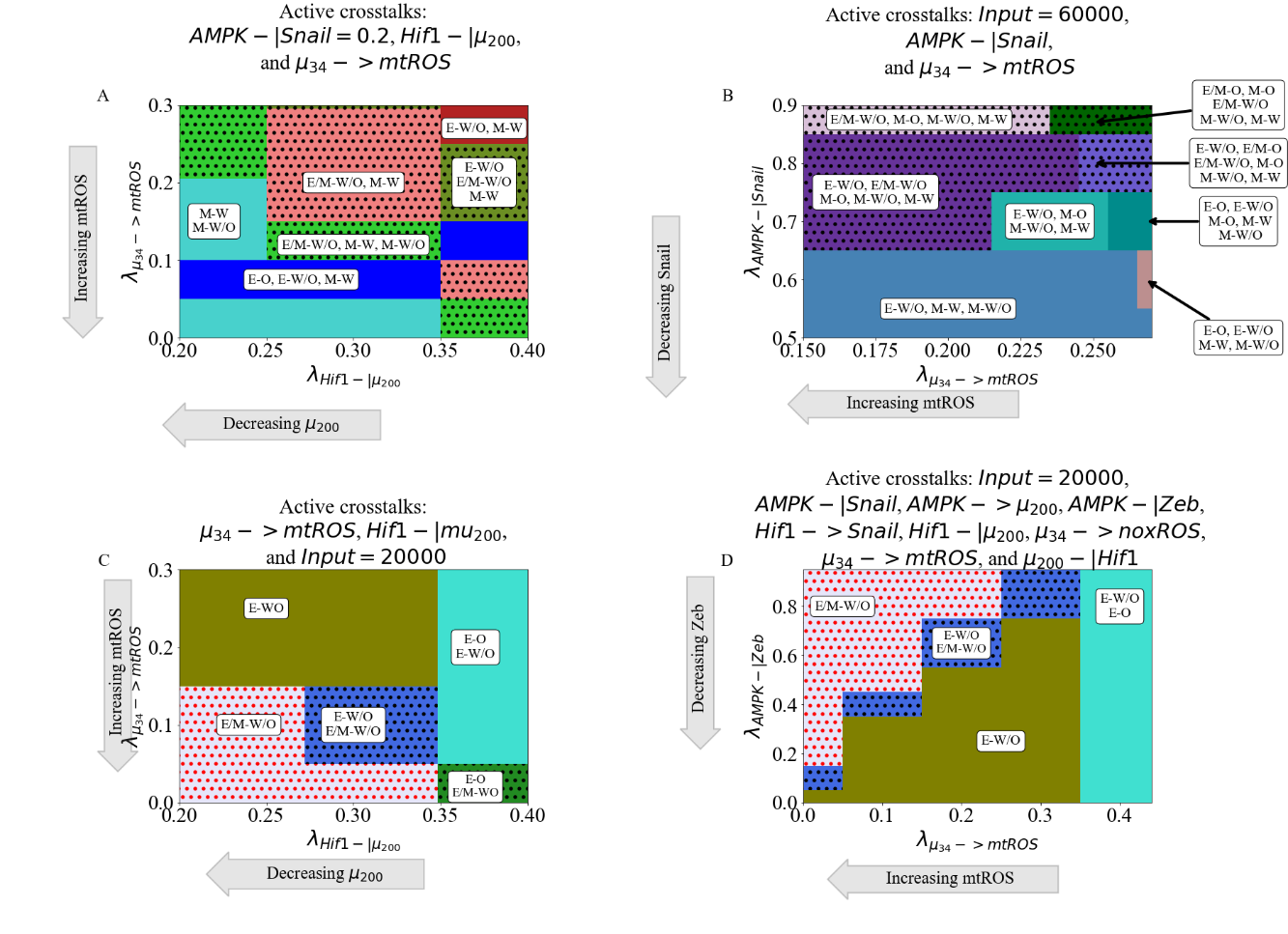
**The Hybrid E/M-W/O phenotype:**

Recently, it has been suggested that the most metastatic cancer phenotype is characterized by the hybrid E/M state and/or the hybrid W/O state,. Therefore, it is now useful to focus our discussion onto how the crosstalk between EMT and metabolism regulatory networks specifically affects the E/M-W/O state and the possibility that it could be the only possible coupled state for certain parameter space. From our analysis of activating individual crosstalks, we deduce that two competing metabolism driven crosstalk and one EMT driven crosstalks would be minimally necessary to fully stabilize the E/M-W/O state and suppress all other coupled states.

In detail, the hybrid E/M-W/O state can be stabilized when AMPK downregulates SNAIL, HIF-1 downregulates , and upregulates mtROS. The E/M-W/O phenotype exists in much of the space and is increased in prevalence when SNAIL is significantly repressed by AMPK (λAMPK-| SNAIL=0.2), mtROS is up-regulated, and levels have been moderatey downregulated (Fig 7A and S19A). Further, instead of HIF-1 downregulating , increasing SNAIL levels can promote the E/M-W/O state to become even more prevalent (Fig. 7B and S19B). While, the E/M-W/O state was stabilized in both cases (Figs 7 A and B), neither was able to fully stabilize the E/M-W/O state and suppress all other states.

It is possible to suppress all states except the coupled E/M-W/O state with just three regulatory links; HIF-1 inhibiting , upregulating mtROS, and modulating the input to SNAIL (Fig. 7C and S19C). In fact, no other combination consisting of only three regulatory links seems to enable only the E/M-W/O state, () Additionally, this region which only includes the E/M-W/O state persists even if all remaining crosstalks are activated (Fig. 7D and S19D).

Looking at the proximal phases next to the one with just E/M-W/O suggests that stabilization of the E/M-W/O state requires mutual activation between metabolic reprogramming and EMT. When the E/M-W/O state can be the only available stable state (Fig. 7C and 7D), the surrounding phases are the same whether only three crosstalks or all crosstalks are active (E-O and E-W/O), suggesting there may be a sequential path to generate the E/M-W/O state. Further, if the E/M-W/O state is not the only allowed state (Fig. 7A and 7B), the surrounding phases include M-coupled states (M-O, M-W/O, and M-W) as well as E-coupled states (E-O and E-W/O). Together the results suggest that to reach the E/M-W/O state for epithelial cancer, first metabolic reprogramming should occur and the E-W/O should be acquired followed by a partial EMT (E/M-W/O). Additionally, the persistence of the E/M-W/O state suggests there might be other combinations of crosstalks that generate phases where only the E/M-W/O state is possible, although it is outside the scope of this manuscript to find all possible combinations of crosstalks that can enable only the hybrid E/M-W/O coupled state. However, based on these results, we would expect HIF-1 suppressing and upregulating mtROS to be prominent among all such combinations.



**Figure 7. The coupling of the EMT and metabolic regulatory networks can enable a coupled hybrid E/M-W/O state.** Minimally, three links (two effecting the metabolic network and one controlling the EMT network) are necessary to suppress all coupled states except the E/M-W/O state. Many combinations of crosstalks can upregulate the E/M-W/O state, but to enable the E/M-W/O state to be the only stable state, three crosstalk are necessary – miR34 upregulating mtROS, HIF-1 inhibiting , EMT-inducing signals that regulating SNAIL. **(A)** Phase digrams of the coupled states when considering three cross-talk - the input=60000, AMPK downregulates SNAIL, and upregulates mtROS. **(B)** The phase disgram of the coupled states when considering the inhibition of SNAIL by AMPK (λAMPK-| SNAIL=0.2), HIF-1 inhibiting μ200, and upregulating mtROS.. **(C)** When considering the bidirectional regulation between EMT and metabolism by the minimally three cross-talk, there are parameter regions in which the only possible coupled state is the E/M-W/O state. **(D)** When considering the bi-directional regulation between EMT and metabolism by all crosstalks, there are regions where only the E/M-W/O state exists. Similar sets of coupled states in (C) and (D) suggest a preferential pathway to drive the system towards the hybrid E/M-W/O coupled state.

**Normal cells can exhibit hybrid properties when crosstalks introduced:**

**The cross-talk can lead to the emergence of hybrid states**

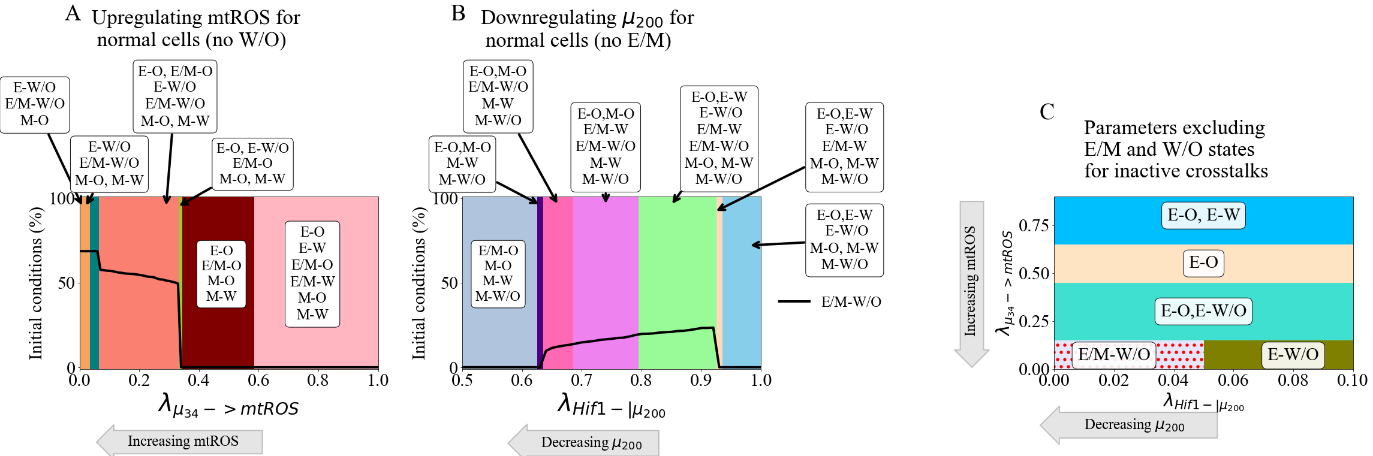
We have showed that the E/M and W/O states are often connected, the population in the E/M-W/O state can be expanded depending on the relative strength of various links, and there are parameter sets with only the hybrid E/M-W/O state available and all other coupled states suppressed. Next, to investigate whether the cross-talk between EMT and metabolism enables cancer plasticity e.g., by acquiring the hybrid states, we simulate senmarios where the individual EMT and the individual metabolism network can’t acquire a hybrid state, then systematically analyze whether any cross-talk can enable the hybrid state to emerge.We confirmed both the EMT and metabolic networks are independently bistable when the crossstalks are inactive ( Fig. S20), and ensured the solutions from the initial conditions were evenly divided between the steady states (Fig. S21-S22). We can then proceed to determine the consequences if EMT and metabolism mutually regulate each other.

As already mentioned, the EMT network can drive metabolic reprogramming via microRNA-mediated links. We first kept the metabolic circuit as a bistable system where only W and O are the available stable states, i.e., no hybrid W/O state when the cross-talk is inactive. Then we analyzed the coupled states considering links - upregulating mtROS, downregulating HIF-1, or upregulating noxROS. We found that the metabolic circuit becomes tristable, i.e., the hybrid W/O state emerges, when mtROS is upregulated by but doesn’t appear Additionally, the upregulation of mtROS by can further stablize the coupledE/M-W/O state so that all E/M phenotypes become coupled with the hybrid W/O phenotype. Furthermore, the upregulation of noxROS in the bistable circuit causes an increase of the frequency of O state, in contrast to an increase of the frequency of the hybrid W/O state in the tristable circuit (). This suggests, noxROS may play a context-dependent role on the coupled state, while mtROS often stabilizes the E/M-W/O phenotype.

Next to see whether metabolic reprogramming can enable the emergence of the hybrid E/M state, we kept the metabolic circuit as a tristable circuit and set the EMT network to be bistable (i.e., unable to acquire a hybrid E/M state alone). Then we investigated the effects of the following cross-talk, HIF-1 inhibiting , HIF-1 upregulating SNAIL, When analyzing the inhibition of by HIF-1, we found that the coupled EMT-metabolism network is able to quickly generate the E/M-W/O state before it is once again suppressed (Fig. 8B). However, the E/M phenotype persists but is coupled to the OXPHOS metabolic phenotype (E/M-O). Additionally, when analyzing the effect of HIF-1 upregulating SNAIL, we found that the E/M-W/O state is only generated when SNAIL is moderately upregualted (Fig. S24). Furthermore, both HIF-1 inhibiting miR-200 and HIF-1 upregulating SNAIL can stabilize the system in the phase - (E/M-O, M-W, M-O, M-W/O), where the hybrid E/M state can be stably maintained. This suggests the master regulator of glycolysis, HIF-1, can drive cells towards the hybrid E/M state. Conversely, an individual AMPK-mediated crosstalk is unable to generate the hybrid E/M state and saturates in the epithelial phase (Fig. S24), as seen in the tristable circuit. Additionally, as with the tristable networks, the coupled hybrid E/M-W/O state can be stabilized by two competing crosstalk, such as AMPK upregulating SNAIL and HIF-1 downregulating (Fig. S25). These results suggest HIF-1 can strongly affect and drive EMT into a hybrid E/M state while AMPK can only help stabilize the E/M-W/O state if it is already present.

When both the EMT and metabolism networks are in the parameter regime where hybrid E/M or hybrid metabolism state is not available, the cross-talk can enable the emergence of these hybrid states. Recall that for the coupled tristable circuits, the simplest set of crosstalk with a parameter region that suppressed all coupled states except the E/M-W/O state consisted of three regulatory links: (1) HIF-1 inhibiting , (2) upregulating mtROS, and (3) EMT-inducing signaling acting on SNAIL. When these same links are active for the bistable EMT and metabolism circuits, the results are qualitatively very similar to the tristable circuit results (Fig. 8C and S26 compared to Fig. 7C). The E/M state is only possible near full inhibition of and the W/O state is possible when mtROS is greatly upregulated. Further, the system must be near maximum regulation (i.e. both fold changes must be close to zero) to generate the region where only the coupled hybrid E/M-W/O state is available. Additionally, the nearby phases surrounding the phase containing only the E/M-W/O state (E-O and E-W/O) is similar relative to those of the tristable circuit, further supporting a preferential pathway that reaches the E/M-W/O state.

Overall, we showed that iwe identified a sequential event to reach the coupled hybrid E/M-W/O state. starting from an E state, which typically associated with an O state, then transitioning from E-O to E-W/O, and finally transitioning to the coupled hybrid E/M-W/O state. In other words, cells probably first reprogram their metabolism from O to the hybrid W/O state, followed by the initiation of EMT and a transition to the hybrid E/M state, thus exhibiting the E/M-W/O state.



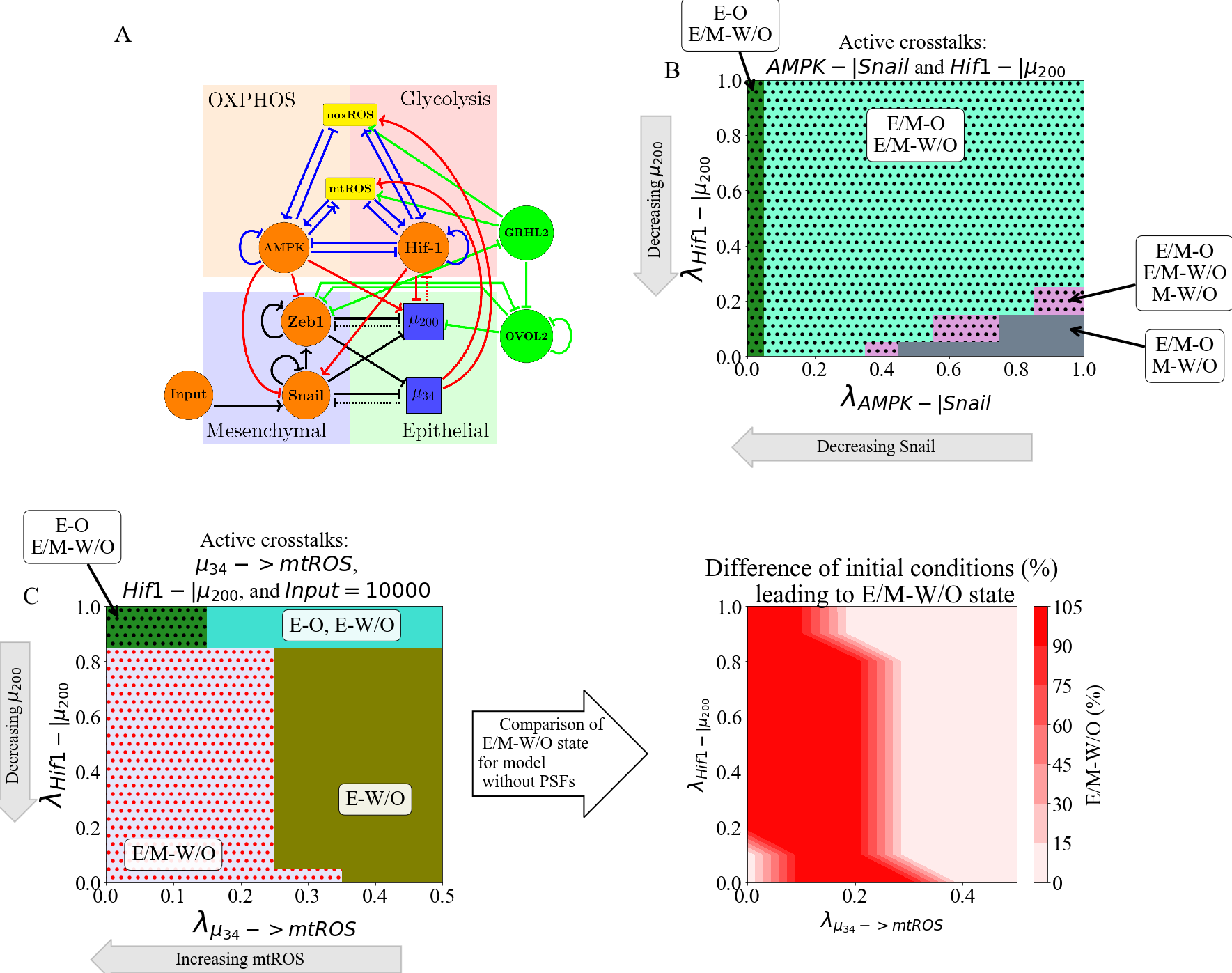
**Figure 8. Crosstalk can generate the hybrid states.** The activation of a single crosstalk (-> mtROS, HIF1 -> SNAIL, or HIF1 -|) can generate the hybrid state of the downstream network (W/O or E/M), respectively.

**OVOL and GRHL2 can stabilize the coupled hybrid E/M-W/O state:**

Previously, we have reported that are transcription factors, such as OVOL and GRHL2 that can stabilize the hybrid E/M state [14,46], referred to as the phenotypic stability factor of the hybrid E/M state. We are curious whether these PSFs of hybrid E/M state may also have a role in stabilizing the coupling of the hybrid E/M state with the hybrid W/O state. Since GRHL2 upregulates ROS in a manner similar to [68], GRHL2 may also stabilize the W/O phenotype. Therefore, we extended the original coupled EMT-metabolism network to include these PSFs (Fig. 9A, parameters and modified equations of the PSF stabilized network are in Section S1.6). When the crosstalks are inactive, we find the PSF stabilized network can either be in the E/M-W/O or E/M-O state, and more than 90% of initial conditions lead to the hybrid E/M-W/O state (Fig. S27).

When a single crosstalk is active in the PSF coupled network, the behavior is as expected, with the E/M-W/O state persisting for larger parameter space relative to the coupled network without the PSFs. For instance, the hybrid E/M-W/O state is the only stable state when one of the following cross-talk is active - AMPK downregulating SNAIL, AMPK upregulation , and upregulating noxROS (see Fig. S28). The parameter space enabling the phase where only the E/M-W/O state is the stable one is also increased when these PSF are present when the following cross-talk is active - HIF-1 downregulating or HIF-1 upregulating SNAIL (Fig. S28).

Next, we studied the effect of the PSFs on the E/M-W/O state when multiple crosstalks are active. If two competing crosstalks acting on the EMT circuit are active (e.g.,, one HIF-1 and one AMPK driven regulation active), then the E/M-W/O state is available for most of the parameter space (Fig. 9B). The regulatory crosstalks controlled by HIF-1 seem to have a stronger affect than the AMPK crosstalks and can push the system towards M, as shown by the presence of the M-W/O state as is inhibited by HIF-1. This corresponds with results from the tristable and bistable coupled networks where AMPK upregulating seems to have a weaker effect, specifically on the stability of the E/M-W/O state, than HIF-1 downregulating . Lastly, we activate all three crosstalks that were shown in the tristable and bistable networks to suppress all states except the E/M-W/O state upregulating mtROS, HIF-1 downregulating , and including the EMT-inducing signal acting on SNAIL. Once again, there is a phase where only the E/M-W/O state can exist (fig?). Furthermore, this phase exists in a far larger region in the presence of the PSFs (the light red region at the bottom left corner of Fig. 9D) relative to in the absence of the PSFs (Fig. 9C and S29), The large red region surrounding the E/M-W/O phase is the additional parameter space where the E/M-W/O state is stabilized when the PSFs are coupled with the network (Fig?). Further, the coupled states in the phases surrounding the region of E/M-W/O are the same as those in the bistable networks (E-W/O and E-O), and also appear in the analysis of the tristable network. These results confirm the PSFs can not only stabilize E/M but also stabilize the coupling of the hybrid E/M state with the hybrid W/O state, i.e., the E/M-W/O state.



**Figure 9. PSFs stabilizing the E/M state can stabilize the associationg of the E/M state with the W/O state. (A)** The coupled EMT-metabolism network including GRHL2 and OVOL2. **(B)** The phase diagram of the coupled states when the PSFs are present. **(C)** The phase diagram of the coupling states when the three cross-talk – EMT-inducing signal acting on SNAIL, upregulating mtROS, and HIF-1 downregulating - are present together. The phase where the E/M-W/O state is the only possible coupled state is significantly enlarged compared to the coupled network without PSFs. **(D)** The difference in the frequency of the E/M-W/O state between (C, PSFs are present) and the original model where the PSFs are absent (Fig?). The dark red region shows the phases in which only the PSF stabilized model can enable the E/M-W/O state. The light red in the bottom left corner near (0,0.1) is the only region in which the E/M-W/O state is the only stable state irrespective of the presence of PSFs. The light red on the right corner is where neither model, PSFs present or not, can generate the E/M-W/O state.

**Discussion**

This work has presented a comprehensive guide to the effects generated by various regulatory links that couple core circuits respectively responsible for the epithelial-mesenchymal transition and the choice of glucose metabolism. We started by considering the activation of individual links and discovered that these can have rather distinct effects. This made it challenging to study the combined effects of simultaneously including several links. This challenge was again compounded by having to consider the external signals that set the parameters of and thereby bias the individual EMT and metabolic subsystems and their interaction. We therefore decided to focus primarily on E/M-W/O phenotypes, as we expect that these cells are the most metastatically capable.

Some of our important findings include:

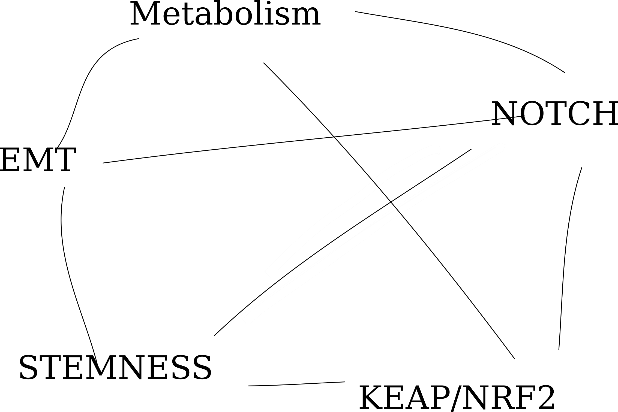
* When the miRNA of the EMT network regulate the metabolic network, the E/M-W/O phenotype can be upregulated with just a single crosstalk. However, when the TFs of the metabolic network regulate the EMT network, a minimum of two crosstalks with opposite effects must be active. Additionally, if crosstalks in both directions are active it is possible to suppress all states except the E/M-W/O phenotype.
* The stabilization of the most plastic E/M-W/O can be facilitated by the addition of “phenotypic stability factors’ (PSFs)\_ to the baseline circuit. Interestingly, one can obtain such states even under conditions when the individual core circuits do not generate hybrid states on their own.
* Lastly, to suppress all coupled states except the hybrid E/M-W/O, our results indicate a progression must be followed; starting from an E-O state, metabolic reprogramming can push the state to E-W/O, followed by partial EMT to stabilize the hybrid E/M-W/O state.

Our results suggest that mtROS is critical to the metabolic activation of EMT. In agreement with our results, recent experimental work has posited that mtROS may drive EMT[69], control cancer invasiveness[70,71], and have a much stronger role than noxROS[48][63]. Also, while it is generally accepted that HIF-1 is important to metabolic reprogramming [22] and triggering EMT[9], the connection between HIF-1, mtROS, and cancer aggressiveness has also been suggested [72]. Indeed, our results suggest the mtROS/HIF-1 axis is critical to stabilizing the highly aggressive E/M-W/O state. Additionally, ROS and HIF-1 expression is controlled by the miRNAs of the EMT network, and , confirming the importance of miRNAs in cancer metastasis [73]. Consequently, our results suggest the existence of a feedback loop between , , HIF-1 and ROS may be critical to stabilizing the E/M-W/O state associated with metastasis and tumorigenesis.

In agreement with other studies[30] our findings indicate that all else being equal, undergoing EMT tends to correlate with using additional glycolysis. This result is consistent with a recent study based on published expression data from public databases[74]. The result is somewhat surprising given the widespread impression that primary tumors often exhibit the Warburg effect, possibly because of their need to limit the amount of ATP produced in favor of maximizing growth (see [75] and references therein). However, this finding is consistent with the general idea that moving from E to E/M is connected with increasing stemness, and stem-like capabilities often rely on glycolysis. Resolution of this issue must await a more precise idea of the phrase ‘all else being equal”. For example, we have ignored external driving of Hif-1 as would clearly occur in hypoxic environments, Mesenchymal cells that reduce proliferation and have to traverse the ECM should switch to more OXPHOS, whereas ones that become quiescent in a hypoxic metastatic niche should favor glycolysis.

In line with the above, this work is merely a first step and it is quite likely that incorporating additional pathways may be necessary to improve our understanding of the mutual activation between EMT, metabolic reprogramming and other physiological factors. One such factor is NRF2. Coupling the KEAP1-NRF2 pathway to Notch signaling has been connected to the E/M phenotype [43], and NRF2 is also an antioxidant that must be downregulated to upregulate ROS production [48–50]. Perhaps the metabolic phenotype of NRF2-stabilized E/M cells may correspond to a hybrid W/O phenotype [29]. Additionally, the p53 pathway seems to upregulate noxROS, and also interferes with EMT [52,53]. Additionally, the E/M-W/O state was stabilized when the Input to SNAIL was modulated confirming the tumor microenvironment and other signals, such as TGF- and NF-, may be important to generating the E/M-W/O state [76,77]. The importance of external signaling in our model is in conceptual agreement with a hypothesis by Sciacovelli and Frezza that, in an adverse tumor microenvironment, metabolic reprogramming drives EMT to allow cells to find more favorable metabolic niches[40]. The importance of the mtROS/HIF-1///SNAIL feedback loop could be experimentally tested by reducing the antioxidant factor SOD2, inducing hypoxia, and treating the cells with NF-.

We believe that understanding how the E/M-W/O phenotype is stabilized by the crosstalks of EMT and metabolic reprogramming could be of vital importance to disrupt metastatic processes. While EMT seems to be able to stabilize metabolically advantageous phenotypes, like the hybrid W/O state, more evidence seems to support a scenario in which metabolic reprogramming drives EMT, especially regarding OXPHOS and glycolysis[32,38,78]. Our results suggest that metabolic reprogramming can indeed drive EMT, but metabolic reprogramming does not have to be complete before EMT begins; this allows the most aggressive E/M-W/O phenotype to be stabilized. Further, to ensure only the E/M-W/O state is accessible, the system seems to first require the E-O state, seen in most cells of the primary tumor[79]. Then the cells undergo metabolic reprogramming while maintaining epithelial characteristics (E-W/O coupled state). Lastly, cells begin EMT and stabilize in the E/M-W/O state, suggesting EMT and metabolic reprogramming are strongly correlated. Strikingly, the prevalence of the E/M-W/O state is increased by these crosstalks regardless of phenotypic availability (i.e., whether the initial system is fully E/M-W/O or only E-O, E-W, M-O, and M-W).



**Figure 10: The coupled networks of cancer metastasis.** (temporary figure – redrawing it and confirming crosstalks first)

The mutual activation of the epithelial-to-mesenchymal transition and metabolic reprogramming stabilizes a highly aggressive E/M-W/O phenotype which may be critical to cancer metastasis. Suppressing all coupled states except the E/M-W/O state requires only three links, suggesting the //HIF-1/ROS/SNAIL axis is a key subset of crosstalks. When these crosstalks are active, our model suggests metabolic reprogramming drives EMT but previous work suggests EMT can drive metabolic reprogramming(cite TGFB EMT paper). This open question may be solved by incorporating the crosstalk of many regulatory networks. The coupling of two networks has shown the various phenotypes associated with therapy resistance, increased metastatic potential, and stem-like properties tend to be correlated[82–84]. However, these couplings also resulted in unexpected behaviors such as the co-localization of hybrid E/M cells[82] and a stemness window that was tunable[83]. Consequently, studying individual gene regulatory network modules, even in the presence of signals, is unable to give a thorough understanding of the network properties. Therefore, to understand how the various phenotypes are correlated, and potentially identify key regulators, multiple networks and crosstalks should be studied concurrently. One potential coupling would be the EMT network, stemness network, metabolic network, Notch-Jagged signaling, KEAP/NRF2 pathway, and the immune-suppresor PD-L1 (Fig. 10). From this expansive network, we expect the hybrid S/R, hybrid E/M, hybrid W/O, immune-evasive properties, and stem-like properties would be correlated and key regulators would be identified.

**Acknowledgements**

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