S100A4 and its Role in Metastasis - Simulations of Knockout and Amplification of Epithelial Growth Factor Receptor and Matrix Metalloproteinases

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Supplementary Material

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1 Text ESI 1

1.1 Principal Component Analysis

Principal component analysis (PCA) is included in our simulation program and is applied to
detect co-variation patterns at the level of steady-state activities of the network components
(equivalent to experimental co-expression patterns) as well as to co-variation of sensitivities
with respect to the applied varying conditions (reflecting co-regulatory patterns). Representa-
tions in Figures ESI 5-8 do not display the network components that were systematically varied
during the simulation procedure. These varied components are however considered in the PCA
computation. Consequently, the dependent variables that are represented in Figures ESI 5-8 are
scaled to the ranges of variation of the independent variables applied in the simulation. The
names of the variables used in the PCA are found in the scheme of Figure 1.

1.2 PCA: S100A4 Knockout

Steady-state values appear not to be influenced by variation in EGFR. Groups associating Cap-
Growth with Plasmin, EphrA1, and a cluster comprising EGFR, NFKB and cytoskeletal pro-
teins maintain stable distance to CellDiss. A cluster composed of CellDiss, CapGrowth, OPN,
Plasmin, uPA, uPAR is observed in the sensitivity PCA in which single variables conserve their
distances. With increasing EGFR, this cluster moves towards a group formed by BCat, EGFR,
NKF and Myo9. ECadh and EphA1 remain distant from the other variables. (See Figure ESI 5).

1.3 PCA: Inhibition of MMPs

Differently from the situation when knocking out S100A4, MMPs inhibition appears to create
barriers between groups of variables. These boundaries increase the separation at higher activity
of EGFR and lead to the formation of groups associating CapGrowth, Plasmin, NFKB, with
EphrA1 and OPN together moving towards the stable variables MMPs and cytoskeletal proteins.
In addition, CellDiss and EGFR move towards each other and swap their relative position at high
EGFR. In the sensitivity PCA three groups are observed. ECadh and Myo9, BCat and MMPs
maintain their positions as EGFR’s activity increases. A third compact group comprising the
remaining variables moves towards the latter one with increasing EGFR. (See Figure ESI 6).

1.4 PCA: S100A4 Knockout and Inhibition of MMPs

The variables’ steady-state is unchanged by increasing NF-κB’s activity from low to medium
with exception of CellDiss and NFKB whose relative distance increases. At high NF-κB the
relative distance of the latter variables increases further and the other variables assume different
pattern with respect to lower levels of NF-κB. Boundaries increasing separation between groups
of variables are observed in the PCA of sensitivity values. CapGrowth, CellDiss and Plasmin
constitute a group closely positioned to another compact cluster formed by OPN, EphrA1 and
uPA, uPAR in the vicinity of NFKB. Relative distances between these groups increase propor-
tionally to NF-κB activity which makes them segregate them apart form each other. (See Figure
ESI 7).
1.5 PCA: Inhibition of EGFR-mediated Feedback of S100A4

A group constituted by $S100A4_{int}$, $S100A4_{ext}$, EGFR, NFKB, BCat, ECadh, Myo9, EphrA1 appears in a S100A4-independent pattern of steady-state values. Three groups formed by ECadh, EphrA1; BCat, $S100A4_{int}$, $S100A4_{ext}$, EGFR, NFKB, Myo9; and CapGrowth, CellDis, Plasmin, uPA, uPAR can be distinguished in the PCA of sensitivities. The latter group moving to the second one by increasing S100A4. (See Figure ESI[8]).

2 Supplementary Figures

Figure ESI 1: S100A4 extended scheme. The nodes added to the S100A4 network shown in Figure 1 are represented in white.

Figure ESI 2: Sensitivity landscapes of the S100A4 extended scheme. Sensitivity of cell dissociation (left) and capillary growth (right). The colour code corresponds to the sensitivity surfaces in Figure 5.
Figure ESI 3: Inhibition of MMPs. Sensitivity of cell dissociation (left) and capillary growth (right). Upper, sensitivity surfaces are calculated in response to variation of S100A4 activity levels ($\varepsilon_{\text{CellDiss S100A4}} = \Delta[\ln(\text{CellDiss})] / \Delta[\ln(\text{S100A4})]$) and are shown in bright colours. The lower surfaces are calculated in response to variation of TIMPs activity levels ($\varepsilon_{\text{CellDiss TIMPs}} = \Delta[\ln(\text{CellDiss})] / \Delta[\ln(\text{TIMPs})]$) and are shown in dark colours overlapping at the zero level of the z-axis (note that the system is independent on TIMPs). EGFR levels correspond to basal activity values set to low = 0.001, medium = 0.01 and high = 0.1.

Figure ESI 4: Combination of S100A4 knockout and inhibition of MMPs. Sensitivity of cell dissociation (left) and capillary growth (right). Upper, sensitivity surfaces are calculated in response to variation of EGFR activity levels ($\varepsilon_{\text{CellDiss EGFR}} = \Delta[\ln(\text{CellDiss})] / \Delta[\ln(\text{EGFR})]$) and are shown in bright colours. Lower surfaces are calculated in response to variation of TIMPs activity levels ($\varepsilon_{\text{CellDiss TIMPs}} = \Delta[\ln(\text{CellDiss})] / \Delta[\ln(\text{TIMPs})]$) and are shown in dark colours overlapping at the zero level of the z-axis (note that the system is independent on TIMPs). NF-κB levels correspond to basal activity values set to low = 0.001, medium = 0.01 and high = 0.1.
Figure ESI 5: S100A4 knockout. Loading plots of MMPs and TIMPs variation. Low (left), medium (middle), high (right) EGFR; upper row: steady-state, lower row: sensitivity.

Figure ESI 6: Inhibition of MMPs. S100A4 and TIMPs variation (loading plots); low (left), medium (middle), high (right) EGFR; upper row: steady-state, lower row: sensitivity.
Figure ESI 7: S100A4 knockout and inhibition of MMPs. EGFR and TIMPs variation (loading plots); low (left), medium (middle), high (right) NF-κB; upper row: steady-state, lower row: sensitivity.

Figure ESI 8: Inhibition of EGFR-mediated feedback of S100A4. MMPs and TIMPs variation (loading plots); low (left), medium (middle), high (right) S100A4; upper row: steady-state, lower row: sensitivity.