

Modeling the dynamics of Let-7-coupled gene regulatory networks linking
cell proliferation to malignant transformation

Claude Gérard^{1,*}, Frédéric Lemaigre¹ and Didier Gonze²

¹de Duve Institute, Université catholique de Louvain, Brussels, Belgium

²Unité de Chronobiologie théorique, Faculté des Sciences,
Université libre de Bruxelles, Brussels, Belgium

*Corresponding author: Claude Gérard (claude.gerard@uclouvain.be)

Keywords: Cdk network; cell cycle; inflammatory circuit; deterministic model; cell population; random switch

Abstract

The microRNA Let-7 controls the expression of proteins that belong to two distinct gene regulatory networks, namely a cyclin-dependent kinases (Cdks) network driving the cell cycle and a cell transformation network which can undergo an epigenetic switch between a non-transformed and a malignant transformed cell state.

Using mathematical modeling and transcriptomic data analysis, we here investigate how Let-7 controls the Cdk-dependent cell cycle network, and how it couples the latter with the transformation network. We also assess the consequence of this coupling on cancer progression.

Our analysis shows that the switch from a quiescent to a proliferative state depends on the relative levels of Let-7 and several cell cycle activators. Numerical simulations further indicate that the Let-7-coupled cell cycle and transformation networks mutually control each other, and our model identifies key players for this mutual control. Transcriptomic data analysis from The Cancer Genome Atlas (TCGA) suggest that the two networks are activated in cancer, in particular in gastrointestinal cancers, and that the activation levels vary significantly among patients affected by a same cancer type. Our mathematical model, when applied to a heterogeneous cell population, suggests that heterogeneity among tumors may in part result from stochastic switches between a non-transformed cell state with low proliferative capability and a transformed cell state with high proliferative property. The model further predicts that Let-7 may reduce tumor heterogeneity by decreasing the occurrence of stochastic switches towards a transformed, proliferative cell state.

In conclusion, we identified the key components responsible for the qualitative dynamics of two networks interconnected by Let-7. The two networks are heterogeneously activated in several cancers, thereby stressing the need to consider patient's specific characteristics to optimize therapeutic strategies.

Introduction

Let-7 microRNAs control two distinct gene regulatory networks (GRNs) that regulate cell cycling and malignant transformation of breast cancer cells (Johnson et al., 2007; Iliopoulos et al., 2009). A cyclin-dependent kinases (Cdk) network controls the correct progression of the cell cycle along the G1, S, G2 and M phases (Morgan, 2007). Growth factors (GF) and E2F stimulate, while Let-7 down-regulates the expression of several components of this Cdk-dependent cell cycle network (Bueno and Malumbres, 2011). Mathematical models focusing on post-translational regulations of cyclin/Cdk complexes were proposed to account for the dynamics of the Cdk network in mammals (Novak and Tyson, 2004; Gerard and Goldbeter, 2009). However, to our knowledge, no model has been proposed to study the impact of miRNAs on this network.

Let-7 is also a key component of a GRN which promotes cell transformation in response to an inflammatory stimulus (Iliopoulos et al., 2009). This GRN is characterized by a positive feedback loop (PFL), where a transient inflammatory stimulus is sufficient to induce the cells to undergo a PFL-dependent epigenetic switch from a non-transformed state toward a permanently malignant transformed state. We previously proposed a model describing the dynamics of this transformation GRN (Gerard et al., 2014). Our model suggested that a transient inflammatory signal activates an irreversible bistable switch in the expression of the GRN components, eventually leading to a stable epigenetic state allowing cells to display increased motility and invasiveness. In this GRN, Let-7 prevents cell transformation by inhibiting the translation of Interleukin-6 (IL6) and Ras, two oncogenic drivers.

Let-7 being a component common to the cell cycle and transformation networks we now raise the following questions: how does Let-7 control the Cdk-dependent cell cycle network; does Let-7 play a coupling role between the cell cycle GRN and the transformation GRN, and can the two GRNs be combined into a larger network that impacts on cancer progression? We address these issues using experiment-based mathematical modeling of the GRNs and by analyzing transcriptomic data from The Cancer Genome Atlas (TCGA). We characterize the qualitative dynamics resulting from the coupling between the two GRNs and show

that their activation is cancer-specific and heterogeneous from patient to patient, stressing the need to consider patient's specific characteristics.

Results

Structure of the cell cycling and transformation networks and model description. The structure of the Cdk network gives rise to a transient and sequential activation of the various cyclin/Cdk complexes, allowing for a correct progression through the different cell cycle phases (Fig. 1A and (Morgan, 2007;Gerard and Goldbeter, 2009)). The activity of cyclin D/Cdk4-6 ensures the transition G0/G1 and the progression in G1. Cyclin E/Cdk2 promotes the G1/S transition, while cyclin A/Cdk2 elicits progression in S and G2. Finally, cyclin B/Cdk1 brings about G2/M transition and the entry of cell into mitosis (Fig. 1A). In the model, Let-7 represses each cyclin/Cdk complex (see next section for details).

Let-7 is also at the core of a positive feedback loop (PFL) in a malignant transformation network (Iliopoulos et al., 2009). Indeed, a transient inflammatory signal mediated by the oncoprotein Src stably activates NF- κ B, which promotes Lin28, IL6 and STAT3 activation (Iliopoulos et al., 2010;Fofaria and Srivastava, 2015). Multiple links exist between cell proliferation and the processes of cell transformation and cell migration (Zhang and Liu, 2002). Indeed, NF- κ B, Lin28, Ras, STAT3 may all regulate cell proliferation, which strengthens the coupling between cell proliferation and cell transformation (Coqueret and Gascan, 2000;Bienvenu et al., 2001;Brantley et al., 2001;Ding et al., 2008;Xiong et al., 2008;Xu et al., 2009). Here, we do not aim to propose a comprehensive mathematical model to analyze the multiple, redundant, intertwined links between both GRNs. We focus on Let-7 and explore its potential role to couple both gene regulatory networks driving proliferation and malignant transformation.

The model is described by a set of 15 kinetic equations for the Cdk network driving the mammalian cell cycle and 14 kinetic equations for the inflammatory circuit that controls the dynamics of malignant transformation (Gerard et al., 2014). Each equation represents the temporal evolution of the expression level of one component of the network. For the cell cycle network, it includes the mRNAs of cyclin D, E, A, and B; the active form of E2F; the various cyclin/Cdk complexes (cyclin D/Cdk4-6, cyclin E/Cdk2, cyclin A/Cdk2 and cyclin B/Cdk1), and the active form of the

Anaphase-Promoting Complex, APC, which triggers degradation of cyclins A and B at the end of mitosis (Fig. S1). We assume that the different complexes cyclin/Cdk are formed immediately after the synthesis of cyclin, Cdk being present in excess, and, consequently, we only consider the synthesis and degradation of these complexes. Variables are defined in Table S1, the kinetic equations are given in Table S3, and the parameters are defined in Table S5.

Relative levels of Let-7 and growth factors control cell cycling. To analyze the impact of Let-7 on the Cdk network dynamics, we built a qualitative skeleton mathematical model of cell cycle regulation by Let-7 (see Figs. 1A for an overview of the model's structure and Fig. S1 for a detailed description highlighting all regulatory interactions included in the model). The model is an extension of an earlier model of the Cdk network that accounted for the dynamics of the mammalian cell cycle (Gerard and Goldbeter, 2011). It now explicitly incorporates the mRNA form of each cyclin, enabling us to describe Let-7-mediated post-transcriptional regulation of cyclin synthesis. Let-7 represses the synthesis of multiple activators of the cell cycle, such as cyclins D, E, A, B, Cdk6, and E2F (see (Bueno and Malumbres, 2011) and Table S2). For the sake of simplicity we consider that Let-7 directly represses the translation of cyclins D, E, A and B, by forming an "inactive" complex with their respective mRNA (once bound to Let-7, cyclin mRNA cannot be translated). In addition, GF promote the synthesis of cyclin D, eliciting the G0/G1 transition and the entry of the cell into the cell cycle, while E2F activates synthesis of cyclins E and A. In this version of the model for the cell cycle, we do not consider the inhibitory impact of RB on cell cycle progression (Giacinti and Giordano, 2006; Gerard and Goldbeter, 2009).

As a consequence of its regulatory structure the network self-organizes with sustained oscillations in the activity of the various cyclin/Cdk complexes, which correspond to successive rounds of cell cycling. The occurrence of the oscillations however depends on the levels of Let-7 and GF. In the absence of both Let-7 and GF, cells proliferate and sustained oscillations of the various cyclin/Cdk complexes develop (Fig. 1B). This situation bears similarity with transformed or cancer cells, which are often characterized by downregulation of Let-7 and signal-independent growth (Sotiropoulou et al., 2009; Hanahan and Weinberg, 2011). Starting from that condition, an increase in GF maintains cell proliferation (Fig. 1C), while an increase

in Let-7 suppresses cell proliferation (Johnson et al., 2007). The latter case is characterized by a stable steady state, with low levels of each cyclin/Cdk (Fig. 1D). Finally, starting from that steady state, an increase in GF permits to recover cell proliferation (Fig. 1E).

In this version of the model for the cell cycle, Let-7 is the only brake for cell cycle progression. Thus, in the absence of Let-7, the Cdk network is able to enter into a sustained oscillatory regime in a GF-independent manner. Because there is a balance between inhibitors and activators of the cell cycle that controls the decision between quiescence and proliferation (Gerard and Goldbeter, 2014), an increase in the level of Let-7 renders the Cdk network dependent on an activator of the cell cycle, here GF, to enter into a sustained oscillatory regime. A previous theoretical study already predicted that a balance between E2F (activator of the cell cycle) and RB (inhibitor of the cell cycle) controls the existence of sustained oscillations of the Cdk network that are either dependent or independent on GF (Gerard and Goldbeter, 2012).

The respective impact of Let-7 and GFs can be visualized in a two-parameter plane where the dynamical behavior of the cell cycle network is represented as a function of the synthesis rate of Let-7, V_{SLET7} , and the level of growth factors, GF (Fig. 2A). For large values of V_{SLET7} (high levels of Let-7), the Cdk network tends to a stable steady state corresponding to cell cycle arrest regardless of GF levels. This corroborates experimental results showing that Let-7 represses cell proliferation (Johnson et al., 2007; Zhu et al., 2015). In contrast, for small values of V_{SLET7} (low levels of Let-7), the cell cycle network is characterized by sustained oscillations. The temporal evolution of cyclin E/Cdk2 and cyclin B/Cdk1 corresponding to conditions A to F in Fig. 2A are represented in Fig. S2A-F, respectively.

Note that we interpret the amplitude of the cyclin/Cdk oscillations in a semi-quantitative manner. We consider that high amplitude of cyclin E/Cdk2 and cyclin B/Cdk1 oscillations triggers cell proliferation by triggering G1/S and G2/M transitions, respectively (Figs. 1-2 and Fig. S2A,C). The sustained oscillations in cyclin E/Cdk2 with oscillations in cyclin B/Cdk1 of very small amplitude might correspond to endoreplication (Edgar et al., 2014) where multiple rounds of DNA replication occur without entry into mitosis (see temporal evolution in Fig. S2D, which corresponds to condition D in Fig. 2A). Previous theoretical studies already showed that the regulatory structure of the cell cycle network in mammals is capable of generating

endocycles (Gerard and Goldbeter, 2009). Near the Hopf bifurcation the oscillations of the various cyclin/Cdk may not have a sufficient amplitude to trigger the transitions into the successive cell cycle phases. However the domain of limit-cycle oscillations nevertheless provides an estimate of the extent of the proliferation domain and how it is affected upon changes in parameter values; it also indicates what would be the impact of changes in control parameters.

The dynamics of the cell cycle network is further illustrated for different levels of Let-7 in a two-parameter plane defined by the synthesis rate of the cyclin/Cdk complexes, V_{SMCYC} , and the level of GF, (Fig. 2B-D). By increasing Let-7, the domain of sustained oscillations, corresponding to cell proliferation, is reduced and limited to higher levels of cyclin/Cdk complexes (compare Fig. 2B, C, D where V_{SLET7} is equal to 0, 0.25 and 3, respectively). From a stable steady state, corresponding to quiescence (condition 1 in Fig. 2C), an increase in GF or an increase in the cyclin/Cdk levels may trigger the switch to sustained oscillations (see temporal evolution of cyclin B/Cdk1 in Fig. 2E and 2F).

We concluded that progression or arrest of the cell cycle is controlled by the relative levels of Let-7 and GF, or of Let-7 and the cyclin/Cdk complexes. Thus, when designing efficient anti-cancer strategies, the model stresses the importance to consider the relative, rather than the absolute, expression levels of network components displaying opposing effects on cell cycling.

Let-7 couples the cell cycling and transformation networks. Let-7 belongs both to the Cdk-dependent cell cycle GRN and the malignant transformation network (Fig. 1A). Therefore, we here determine the qualitative role of Let-7 as a coupling factor between the two GRNs, by analyzing the mutual impact of the GRNs on their respective dynamics.

Starting from a non-transformed, quiescent, cell state, defined by high Let-7 and low cyclin B/Cdk1 levels, a transient Src signal induced by inflammation triggers a down-regulation of Let-7, eliciting the switch of the cell cycle network from a stable steady state to sustained oscillations (Fig. 3A). Thus, transient inflammatory signals can promote persistent cell proliferation. On the opposite, as observed in the experiments (Iliopoulos et al., 2009), starting from a transformed and proliferative cell state, transient inhibition of Lin28, NF- κ B or transient overexpression of PTEN stably impedes cell cycle progression (Fig. 3B-D).

We concluded that transient modifications in the expression of the components of the inflammation-dependent bistable transformation network can impact the long-term behavior of the cell cycling network when Let-7 couples the two GRNs.

To determine if the cell cycle network can modulate the dynamics of the transformation network, we simulated overexpression of all cyclins by increasing their synthesis rates, via the parameter V_{SMCYC} . Cyclin overexpression promotes uncontrolled cell proliferation of cancer cells (Gillett et al., 1994; Pok et al., 2013). Starting from a non-transformed cell state (high Let-7 levels), the model shows a down-regulation of Let-7 when V_{SMCYC} increases (Fig. 4A where V_{SMCYC} increases at $t=100h$). The corresponding temporal evolution of Let-7 and cyclin B/Cdk1 is represented for different synthesis rates of cyclins (at $t=100h$, V_{SMCYC} changes from 1.5 to 2.5 in Fig. 4B and from 1.5 to 12 in Fig. 4C). For weak overexpression of cyclins, the non-transformed quiescent cell state is maintained, while for stronger cyclin overexpression both GRNs are activated, i.e. downregulation of Let-7 and sustained oscillations of the cell cycle network (Fig. 4B-C). Note that for mild overexpression of cyclin, the time needed for Let-7 downregulation is rather long, possibly longer than the life time of the cell. It is thus likely that the cell will not undergo a transition towards division. However, in the presence of large cyclin/Cdk overexpression (Fig. 4C), the time needed for the switch to occur is much shorter.

Temporal evolution of Let-7 and cyclin B/Cdk1 indicates that the switch to cell proliferation triggered by cyclin overexpression is irreversible because down-regulation of cyclins to their initial levels will not restore cell cycle arrest (Fig. S3A, condition 1, $1200h < t < 1500h$). This is the consequence of the irreversible bistable switch at the core of the transformation GRN (Gerard et al., 2014). The model predicts that a stronger decrease in cyclin synthesis can eventually stop cell proliferation, characterized by low, stable steady state levels of cyclin B/Cdk1 (Fig. S3A, condition 3, $t > 1500h$). The corresponding temporal evolution of the expression levels of Lin28 and STAT3, two critical activators of the transformation GRN, is shown in Fig. S3B. The model indicates that these cells (condition 3 for $t > 1500h$) might be invasive cells, defined by high levels of STAT3 and Lin28 and low Let-7 levels, which are in a quiescent state (low levels of cyclin/Cdk).

Moreover, the model predicts that a transient down-regulation of Lin28 can impede both cell proliferation and the transformation network (Fig. S3C, condition 3, t

> 1500h), where low, stable, levels of cyclin B/Cdk1 are present with high Let-7 levels. The corresponding temporal evolution of Lin28 and STAT3 expression levels is represented in Fig. S3D. Here also, modeling predictions correlated well with the experimental observations showing downregulation of both cell proliferation and cell transformation after transient inhibition of Lin28 (Iliopoulos et al., 2009).

Growth factors may also couple the transformation and cell cycling networks.

After binding to specific ligands, tyrosine kinase receptors can lead to the activation of Ras (Schlessinger, 2000). Thus in cells which possess active tyrosine kinase pathways, we expect that growth factors simultaneously activate cell proliferation, Ras and the transformation network. The resulting coupling mechanisms between the proliferation and the transformation networks should thus be reinforced. We simulated the coupling mechanisms of the two networks via active tyrosine kinase signaling (Fig. S5). The model indicates that, even in the absence of Src (the triggering signal of the transformation network), GF is able to activate both the proliferation and the transformation networks. The model predicts that the network activation is faster for high levels of GF (compare Fig. S5B with S5C). Depending on cell types, GF may lead to the activation of cell proliferation through numerous different signaling pathways that do not necessarily depend on Ras. In the latter cells, we may thus suppose that GF activate cell proliferation without affecting the transformation network via Ras. In the following we do not take into account GF-mediated Ras-activation.

Cancer type-specific activation of the proliferation and transformation networks. The coupling of the two networks raised the question of their potential combined involvement in cancer. To address this issue, we first defined a Cell Proliferation Index (*CPI*) and a Non-Transformed State Index (*NTSI*), where:

$$CPI = \max(Md) + \max(Me) + \max(Ma) + \max(Mb)$$

and

$$NTSI = \frac{PTEN + Let7}{IL6 + NFkB + STAT3 + miR21 + Lin28 + Ras}$$

CPI is the sum of the maximal RNA levels of all cyclin/Cdk complexes, which is, as a first approximation, an indication of cell proliferation; *NTSI* is the ratio between the RNA expression levels of the inhibitors, i.e. Let-7 and PTEN, divided by the expression levels of the activators of the epigenetic transformation switch, i.e. IL6, NF- κ B, STAT3, miR21, Lin28 and Ras. Such ratio characterizes the degree of cell transformation where a high value defines a non-transformed cell while a low value corresponds to transformed cells. We here make use of the available gene expression data (TCGA) to measure CPI and NTSI in various cancers.

In addition, because the cell cycle is driven by the activity of successive cyclin-dependent kinases and since mRNA and protein expression levels are not necessarily positively correlated, we collected, when available, the protein expression levels of the network components from the Cancer Proteome Atlas (<https://tcpaportal.org/tcpa/index.html>). We showed that the mRNA and protein expression levels of CYCLIN B1, a major component driving mitosis entry, are positively correlated in the two studied cohorts, namely hepatocellular carcinoma (HCC) and prostate adenocarcinoma (Fig. S6A,B). This already supports the choice of CPI, which is based on mRNA levels, as an indicator of cell proliferation.

To further support our indexes, we performed a Principal Component Analysis (PCA) based on the mRNA expression levels of 124 components belonging to the cell cycle network pathway as defined in the Kyoto Encyclopedia of Genes and Genomes, KEGG (<https://www.genome.jp/kegg/pathway.html>) (Fig. S6C). This PCA analysis performed on the HCC cohort (TCGA) permits to select 100 HCC samples with high cell cycle activation (red dots in Fig. S6C) and the other HCC samples defined as low cell cycle activation (grey dots). Indeed, HCC with high cell cycle activation are located at the farthest position as compared to non tumor samples (blue dots). CPI are higher in HCC with high cell cycle activation compared to HCC with low cell cycle activation (Fig. S6D), which validates CPI as a good proxy of cell proliferation.

In addition, CPI measured in 20 HCC cell lines (RNASeq data from the OASIS Genomics portal: <http://www.oasis-genomics.org/>) is much higher than CPI in HCC, which fits with the large cell proliferation capability of immortalized cell lines as compared to tumor samples (Fig. S6D).

Furthermore, in this work, the expression levels of Let-7 and cyclin B/Cdk1 were repeatedly used as markers of cell transformation and cell proliferation states

(Figs. 3 and 4). As a support of this, we verified that in liver cancer, the expression of Let-7 and CYCLIN B1 mRNA are negatively correlated, in a similar manner, than *NSTI* versus *CPI* (Fig. S6E,F).

In conclusion, these analyses support the choice of *CPI* and *NSTI* as good indicators of cell proliferation and cell transformation states.

Since the components of the cell cycling and transformation networks are expressed in several tissues we assessed if the networks are activated, i.e. if their components are consistently misexpressed in different types of cancer. We first calculated *CPI* and *NSTI* based on the RNA expression of the network components (Table S7) from the non tumor (NT) and tumor samples (T) of TCGA cohorts of cholangiocarcinoma (CHOL: NT=9, T=36), stomach and esophageal carcinoma (STES: NT=46, T=600), hepatocellular carcinoma (LIHC: NT=50, T=369), stomach adenocarcinoma (STAD: NT=35, T=415), lung squamous cell carcinoma (LUSC: NT=51, T=501), bladder urothelial carcinoma (BLCA: NT=19, T=408), kidney renal clear cell carcinoma (KIRC: NT=72, T=534), breast carcinoma (BRCA: NT=112, T=1100), thyroid carcinoma (THCA: NT=57, T=510), kidney renal papillary cell carcinoma (KIRP: NT=32, T=290), kidney chromophobe (KICH: NT=25, T=66), and prostate adenocarcinoma (PRAD: NT=52, T=498) (Fig. 5A, B). Significant and consistently high *CPI* and low *NSTI* values, as compared to non-tumor conditions, were obtained in several cancer types, with largest variations in gastrointestinal cancers, i.e. cholangiocarcinoma, hepatocellular and stomach and esophageal carcinoma. Interestingly, the proliferation and transformation networks do not seem to be activated in kidney and prostate adenocarcinoma.

This suggests that concomitant activation of the cell proliferation and transformation networks is cancer type-specific and predominantly occurs in gastrointestinal cancers.

Patient-to-patient heterogeneity in the activation of the proliferation and transformation networks. Principal component analysis (PCA) based on the expression of all network components was performed in three cohorts from TCGA: (1) the cholangiocarcinoma cohort, which displays the highest *CPI* levels; (2) the hepatocellular carcinoma cohort, which shows high *CPI* and low *NSTI* values, and (3) the prostate adenocarcinoma cohort characterized by low *CPI* and high *NSTI*. This analysis revealed that non-tumor (blue dots) and tumor samples (red dots) cluster

separately in cholangiocarcinoma (Fig. 6A), suggesting that the combined expression of the components of both networks can be used as a proxy to determine the tumorigenic state of a sample in this cohort.

A larger level of heterogeneity was detected within the hepatocellular carcinoma and prostate adenocarcinoma cohorts (Fig. 6B-C). Moreover, some tumors cluster together with normal samples indicating that the networks are not activated in all samples. Also, despite that the mean *CPI* and *NTSI* values of prostate adenocarcinoma do not significantly differ from those in corresponding non-tumor samples (Fig. 5A, B), some individual tumors are characterized by high activation of the networks (Fig. 6C).

We concluded that the network activation varies from patient to patient and depends on tumor-type.

Dynamics of cell proliferation and cell transformation of a heterogeneous cell population. To determine the source of heterogeneity observed in patient samples, we incorporated a stochastic source of heterogeneity in a cell population model by applying, for each cell, uniform random variations around the basal value of each kinetic parameter. We plotted the simulated levels of *CPI* as a function of *NTSI* in a heterogeneous cell population of quiescent, non-transformed cells (Fig. 7A, C, E; orange dots correspond to *CPI* and *NTSI* in the absence of random variations on parameter's values), and in a population of transformed, proliferative cells (Fig. 7B, D, F; green dots correspond to *CPI* and *NTSI* in the absence of random variations). 10%, 25% and 50% of uniform random variations on each parameter were considered.

Simulations indicate that the non-transformed, quiescent, cell state is less robust to random fluctuations than the transformed, proliferative, state (compare Figs. 7C with 7D, and 7E with 7F). Indeed, starting in a non-transformed, quiescent, cell state, a large proportion of cells switch to a transformed, proliferative, state in the presence of random fluctuations in kinetic parameters (Fig. 7C and 7E). However, from a transformed, proliferative, cell state, only a small proportion of cells switch to a quiescent and non-transformed state (Fig. 7F).

Thus, random fluctuations in kinetic parameter values could trigger abrupt switches in the dynamics of the cell cycling and transformation networks. The “non-tumor” state (quiescent, non-transformed cells) is more sensitive to random

fluctuations in kinetic parameter values than “tumor” state (transformed, proliferative state).

We next assessed if the levels of key network components, i.e. Let-7, PTEN, or Ras, may affect the robustness of these cell states. In a heterogeneous population of non-transformed, quiescent, cells with 25% of random parameter variations (Figs. S7A-F), an increase in Let-7 strengthens the robustness of the non-transformed, quiescent, cell state towards random fluctuations in kinetic parameters and prevents random switches to a transformed, proliferative, cell state (compare basal conditions in Fig. S7A with Figs. S7B, C, D where V_{SLET7} changes from 10 to 12, 20, and 50, respectively). Similarly, an increase in PTEN or a decrease in Ras also improved the robustness of the non-transformed, quiescent, cell state (compare Figs. S7A with S7E and S7F).

Along the same lines, tumor suppressors and oncogenes can also impact on the robustness of the transformed, proliferative, cell state (Fig. S7G-L). Some cells revert to a non-transformed, quiescent, state following a large increase in Let-7 (compare Fig. S7G with S7H, I, J). However, an increase in PTEN or a decrease in Ras (similar to the conditions in Figs. S7E and S7F) are unable to revert cells to a non-transformed, quiescent, state (compare Fig. S7G with Figs. S7K and S7L).

Thus, an increase in tumor suppressors or a decrease in oncogenes reduces the probability of stochastic switches to a transformed, proliferative, cell state. However, if cells are already in a proliferative, transformed state, similar changes in tumor suppressors or oncogenes do not permit to revert back to a more “healthy” cell phenotype, which highlights an irreversible process in cancer progression.

Finally, since cholangiocarcinomas are characterized by strong network activation (Figs. 5 and 6A), we analyzed if the cell population model can qualitatively reproduce the networks' switch from normal to tumor condition. Plotting cyclin B1 mRNA as a function of cyclin E1 or Let-7c RNA (Fig. 8A-D), and plotting Kras mRNA (representative as Ras) as a function of Let-7c (Fig. 8E, F) revealed expression patterns that are qualitatively very similar to those predicted by the mathematical model of a heterogeneous cell population (Fig. 8B, D, E). We concluded that the cell population model can be used to assess the qualitative dynamics of the switch of both networks in cholangiocarcinomas.

Discussion

Tumorigenesis rests on many biological features which include sustained proliferative signaling, evading growth suppressors, resisting cell death, promoting angiogenesis, ensuring replicative immortality and eliciting invasion and metastasis (Hanahan and Weinberg, 2011). Here, we built a mathematical model to analyze the dynamical properties of a Let-7-dependent mechanism coupling cell proliferation and an epigenetic switch driving malignant transformation.

Our mathematical model illustrates qualitatively how Cdk-dependent and transformation networks may interact, and proposes a mechanism, acting through Let-7, which suggests that cyclin overexpression can promote cell proliferation, while inducing and accelerating malignant transformation. Indeed, overexpression of cyclins progressively sponges the free form of Let-7. The latter will no longer be available to repress the components of the transformation network, leading to the activation of the epigenetic switch. This effect is known as competing-endogenous, ceRNA, effect. CeRNAs regulates other RNA transcripts by competing for shared miRNA and were involved in tumorigenesis (Salmena et al., 2011; Tay et al., 2014; Chiu et al., 2017). Here Let-7 is the shared miRNA between both networks. Let-7 was shown to be involved in different ceRNA mechanisms. Indeed, Let-7e can modulate the inflammatory response in vascular endothelial cells through a ceRNA effect (Lin et al., 2017). Imprinted H19 lncRNA, which plays important roles in development, cancer and metabolism, modulates Let-7 availability by acting as a molecular sponge and causing precocious muscle differentiation (Kallen et al., 2013). Moreover, amplification of MYCN mRNA levels in neuroblastoma can sponge Let-7 thereby rendering LIN28B dispensable for cancer progression (Powers et al., 2016). Note however that experimental and theoretical studies indicate that a ceRNA effect between multiple RNA transcripts and the shared miRNA is effective only in the presence of adequate expression levels of the transcripts and the miRNA (Gerard and Novak, 2013; Denzler et al., 2014; Tay et al., 2014).

Our mathematical model further shows that stochastic variations in kinetic parameter values from cell to cell can create large fluctuations in the global network dynamics, possibly leading to stochastic switches of some cells to a transformed and proliferative state. To better analyze if tumor heterogeneity may be explained by cell-to-cell stochastic switches in the network dynamics, cohorts of single-cell transcriptomic data of tumors would be useful. Stochastic switches in the GRN

dynamics could be a source of heterogeneity in cancer cell populations (Tang, 2012;Patel et al., 2014). Stochastic switches in gene networks were identified in hematopoietic tumor stem cells (Dingli et al., 2007), in the appearance of mammary tumor in mice (Bouchard et al., 1989), and in the differentiation and maturation of T lymphocytes (Davis et al., 1993). These switches in single-cell behaviors may also promote phenotypic equilibrium in population of cancer cells (Gupta et al., 2011). Transcriptomic analysis of TCGA data suggests that the coupling between the cell cycle and a malignant transformation networks, and the activation of these networks in tumors are cancer type-specific, with predominant activation in gastrointestinal cancers. Our PCA analysis reveals inter-patient heterogeneity in network activation in tumors, which stresses the need to consider patient-specific characteristics when optimizing therapeutic strategies aiming to reverse the network dynamics of activated GRNs (Biankin et al., 2015). Inter-patient heterogeneity in the activation of two networks might be the consequence of different tumor developmental stages in each patient. In addition, since each tumor is characterized by intra-tumoral heterogeneity, the location of the tumor biopsy within each patient may also generate heterogeneity in the network activation.

In conclusion, by means of transcriptomic data analysis and modeling-based investigations, we identified a Let-7-dependent connection between two major GRNs involved in tumorigenesis, and whose activation is cancer- and patient-specific. In the future it will be interesting to incorporate in the model the additional regulatory links between the transformation network and the cell cycle (Coqueret and Gascan, 2000;Bienvenu et al., 2001;Brantley et al., 2001;Ding et al., 2008;Xiong et al., 2008;Xu et al., 2009) and to examine their contribution in the dynamics resulting from the coupling between the two networks and in particular in the propensity of the cell to switch into a proliferative, potentially cancerous, state. We anticipate that a better characterization of the dynamics resulting from the combination of other GRNs specific for each patient will help providing a global GRN activation map for personalizing and optimizing cancer treatment.

Acknowledgments

This work was supported by the “Fonds National de la Recherche Scientifique” (FNRS, Belgium) [Convention no T.0015.16 (26021674)].

References

- Biankin, A.V., Piantadosi, S., and Hollingsworth, S.J. (2015). Patient-centric trials for therapeutic development in precision oncology. *Nature* 526, 361-370.
- Bienvenu, F., Gascan, H., and Coqueret, O. (2001). Cyclin D1 represses STAT3 activation through a Cdk4-independent mechanism. *J Biol Chem* 276, 16840-16847.
- Bouchard, L., Lamarre, L., Tremblay, P.J., and Jolicoeur, P. (1989). Stochastic appearance of mammary tumors in transgenic mice carrying the MMTV/c-neu oncogene. *Cell* 57, 931-936.
- Brantley, D.M., Chen, C.L., Muraoka, R.S., Bushdid, P.B., Bradberry, J.L., Kittrell, F., Medina, D., Matrisian, L.M., Kerr, L.D., and Yull, F.E. (2001). Nuclear factor-kappaB (NF-kappaB) regulates proliferation and branching in mouse mammary epithelium. *Mol Biol Cell* 12, 1445-1455.
- Bueno, M.J., and Malumbres, M. (2011). MicroRNAs and the cell cycle. *Biochim Biophys Acta* 1812, 592-601.
- Chiu, H.S., Martinez, M.R., Bansal, M., Subramanian, A., Golub, T.R., Yang, X., Sumazin, P., and Califano, A. (2017). High-throughput validation of ceRNA regulatory networks. *BMC Genomics* 18, 418.
- Coqueret, O., and Gascan, H. (2000). Functional interaction of STAT3 transcription factor with the cell cycle inhibitor p21WAF1/CIP1/SDI1. *J Biol Chem* 275, 18794-18800.
- Davis, C.B., Killeen, N., Crooks, M.E., Raulet, D., and Littman, D.R. (1993). Evidence for a stochastic mechanism in the differentiation of mature subsets of T lymphocytes. *Cell* 73, 237-247.
- Denzler, R., Agarwal, V., Stefano, J., Bartel, D.P., and Stoffel, M. (2014). Assessing the ceRNA hypothesis with quantitative measurements of miRNA and target abundance. *Mol Cell* 54, 766-776.
- Ding, B.B., Yu, J.J., Yu, R.Y., Mendez, L.M., Shaknovich, R., Zhang, Y., Cattoretti, G., and Ye, B.H. (2008). Constitutively activated STAT3 promotes cell proliferation and survival in the activated B-cell subtype of diffuse large B-cell lymphomas. *Blood* 111, 1515-1523.
- Dingli, D., Traulsen, A., and Pacheco, J.M. (2007). Stochastic dynamics of hematopoietic tumor stem cells. *Cell Cycle* 6, 461-466.
- Edgar, B.A., Zielke, N., and Gutierrez, C. (2014). Endocycles: a recurrent evolutionary innovation for post-mitotic cell growth. *Nat Rev Mol Cell Biol* 15, 197-210.
- Fofaria, N.M., and Srivastava, S.K. (2015). STAT3 induces anoikis resistance, promotes cell invasion and metastatic potential in pancreatic cancer cells. *Carcinogenesis* 36, 142-150.
- Gerard, C., and Goldbeter, A. (2009). Temporal self-organization of the cyclin/Cdk network driving the mammalian cell cycle. *Proc Natl Acad Sci U S A* 106, 21643-21648.

- Gerard, C., and Goldbeter, A. (2011). A skeleton model for the network of cyclin-dependent kinases driving the mammalian cell cycle. *Interface Focus* 1, 24-35.
- Gerard, C., and Goldbeter, A. (2012). From quiescence to proliferation: Cdk oscillations drive the mammalian cell cycle. *Front Physiol* 3, 413.
- Gerard, C., and Goldbeter, A. (2014). The balance between cell cycle arrest and cell proliferation: control by the extracellular matrix and by contact inhibition. *Interface Focus* 4, 20130075.
- Gerard, C., Gonze, D., Lemaigre, F., and Novak, B. (2014). A model for the epigenetic switch linking inflammation to cell transformation: deterministic and stochastic approaches. *PLoS Comput Biol* 10, e1003455.
- Gerard, C., and Novak, B. (2013). microRNA as a potential vector for the propagation of robustness in protein expression and oscillatory dynamics within a ceRNA network. *PLoS One* 8, e83372.
- Giacinti, C., and Giordano, A. (2006). RB and cell cycle progression. *Oncogene* 25, 5220-5227.
- Gillett, C., Fantl, V., Smith, R., Fisher, C., Bartek, J., Dickson, C., Barnes, D., and Peters, G. (1994). Amplification and overexpression of cyclin D1 in breast cancer detected by immunohistochemical staining. *Cancer Res* 54, 1812-1817.
- Gupta, P.B., Fillmore, C.M., Jiang, G., Shapira, S.D., Tao, K., Kuperwasser, C., and Lander, E.S. (2011). Stochastic state transitions give rise to phenotypic equilibrium in populations of cancer cells. *Cell* 146, 633-644.
- Hanahan, D., and Weinberg, R.A. (2011). Hallmarks of cancer: the next generation. *Cell* 144, 646-674.
- Iliopoulos, D., Hirsch, H.A., and Struhl, K. (2009). An epigenetic switch involving NF-kappaB, Lin28, Let-7 MicroRNA, and IL6 links inflammation to cell transformation. *Cell* 139, 693-706.
- Iliopoulos, D., Jaeger, S.A., Hirsch, H.A., Bulyk, M.L., and Struhl, K. (2010). STAT3 activation of miR-21 and miR-181b-1 via PTEN and CYLD are part of the epigenetic switch linking inflammation to cancer. *Mol Cell* 39, 493-506.
- Johnson, C.D., Esquela-Kerscher, A., Stefani, G., Byrom, M., Kelnar, K., Ovcharenko, D., Wilson, M., Wang, X., Shelton, J., Shingara, J., Chin, L., Brown, D., and Slack, F.J. (2007). The let-7 microRNA represses cell proliferation pathways in human cells. *Cancer Res* 67, 7713-7722.
- Kallen, A.N., Zhou, X.B., Xu, J., Qiao, C., Ma, J., Yan, L., Lu, L., Liu, C., Yi, J.S., Zhang, H., Min, W., Bennett, A.M., Gregory, R.I., Ding, Y., and Huang, Y. (2013). The imprinted H19 lncRNA antagonizes let-7 microRNAs. *Mol Cell* 52, 101-112.
- Lin, Z., Ge, J., Wang, Z., Ren, J., Wang, X., Xiong, H., Gao, J., Zhang, Y., and Zhang, Q. (2017). Let-7e modulates the inflammatory response in vascular endothelial cells through ceRNA crosstalk. *Sci Rep* 7, 42498.
- Morgan, D.O. (2007). *The cell cycle : principles of control*. London: New Science Press Ltd in association with Oxford University Press.
- Novak, B., and Tyson, J.J. (2004). A model for restriction point control of the mammalian cell cycle. *J Theor Biol* 230, 563-579.
- Patel, A.P., Tirosh, I., Trombetta, J.J., Shalek, A.K., Gillespie, S.M., Wakimoto, H., Cahill, D.P., Nahed, B.V., Curry, W.T., Martuza, R.L., Louis, D.N., Rozenblatt-Rosen, O., Suva, M.L., Regev, A., and Bernstein, B.E. (2014). Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma. *Science* 344, 1396-1401.

- Pok, S., Wen, V., Shackel, N., Alsop, A., Pyakurel, P., Fahrner, A., Farrell, G.C., and Teoh, N.C. (2013). Cyclin E facilitates dysplastic hepatocytes to bypass G1/S checkpoint in hepatocarcinogenesis. *J Gastroenterol Hepatol* 28, 1545-1554.
- Powers, J.T., Tsanov, K.M., Pearson, D.S., Roels, F., Spina, C.S., Ebright, R., Seligson, M., De Soysa, Y., Cahan, P., Theissen, J., Tu, H.C., Han, A., Kurek, K.C., Lapier, G.S., Osborne, J.K., Ross, S.J., Cesana, M., Collins, J.J., Berthold, F., and Daley, G.Q. (2016). Multiple mechanisms disrupt the let-7 microRNA family in neuroblastoma. *Nature* 535, 246-251.
- Salmena, L., Poliseno, L., Tay, Y., Kats, L., and Pandolfi, P.P. (2011). A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? *Cell* 146, 353-358.
- Schlessinger, J. (2000). Cell signaling by receptor tyrosine kinases. *Cell* 103, 211-225.
- Sotiropoulou, G., Pampalakis, G., Lianidou, E., and Mourelatos, Z. (2009). Emerging roles of microRNAs as molecular switches in the integrated circuit of the cancer cell. *RNA* 15, 1443-1461.
- Tang, D.G. (2012). Understanding cancer stem cell heterogeneity and plasticity. *Cell Res* 22, 457-472.
- Tay, Y., Rinn, J., and Pandolfi, P.P. (2014). The multilayered complexity of ceRNA crosstalk and competition. *Nature* 505, 344-352.
- Tyson, J.J., Chen, K.C., and Novak, B. (2003). Sniffers, buzzers, toggles and blinkers: dynamics of regulatory and signaling pathways in the cell. *Curr Opin Cell Biol* 15, 221-231.
- Xiong, H., Zhang, Z.G., Tian, X.Q., Sun, D.F., Liang, Q.C., Zhang, Y.J., Lu, R., Chen, Y.X., and Fang, J.Y. (2008). Inhibition of JAK1, 2/STAT3 signaling induces apoptosis, cell cycle arrest, and reduces tumor cell invasion in colorectal cancer cells. *Neoplasia* 10, 287-297.
- Xu, B., Zhang, K., and Huang, Y. (2009). Lin28 modulates cell growth and associates with a subset of cell cycle regulator mRNAs in mouse embryonic stem cells. *RNA* 15, 357-361.
- Zhang, W., and Liu, H.T. (2002). MAPK signal pathways in the regulation of cell proliferation in mammalian cells. *Cell Res* 12, 9-18.
- Zhu, X., Wu, L., Yao, J., Jiang, H., Wang, Q., Yang, Z., and Wu, F. (2015). MicroRNA let-7c Inhibits Cell Proliferation and Induces Cell Cycle Arrest by Targeting CDC25A in Human Hepatocellular Carcinoma. *PLoS One* 10, e0124266.

Figure legends

Figure 1. Let-7 and GF control cell cycle progression. (A) Scheme of both GRNs coupled by Let-7. (B-E) Temporal evolution of Let-7, cyclin E/Cdk2, cyclin A/Cdk2 and cyclin B/Cdk1 are shown in the absence ($V_{\text{SLET7}} = 0$ in B, C) or in the presence of Let-7 ($V_{\text{SLET7}} = 0.5$ in D, E), and in the absence ($GF = 0$ in B, D) or in the presence of GF ($GF = 10$ in C, E). Parameter values are as in Table S5. A detailed scheme of both GRNs that includes all regulations considered in the model is in Fig. S1.

Figure 2. A balance between Let-7 and cell cycle activators determines the quiescence and proliferative state. (A) The Cdk network dynamics, i.e. sustained oscillations *versus* stable steady states, is represented in a two-parameter plane defined by the synthesis rate of Let-7, V_{SLET7} , and the levels of GF. The Cdk network dynamics are indicated in a two-parameter plot as a function of the synthesis rate of the different cyclins, V_{SMCYC} , and the levels of GF (B) in the absence of Let-7, $V_{\text{SLET7}} = 0$, (C) in the presence of intermediate, $V_{\text{SLET7}} = 0.25$, or (D) high Let-7 levels, $V_{\text{SLET7}} = 3$. (E, F) Temporal evolution of cyclin B/Cdk1 in the presence of GF overexpression (panel E where GF changes from 0.2 to 8, at $t = 100\text{h}$, which corresponds to the switch from conditions 1 to 2 in panel C), or in the presence of cyclin overexpression (panel F where V_{SMCYC} changes from 1 to 4, at $t = 100\text{h}$, which corresponds to the switch from 1 to 3 in panel C). Temporal evolution of cyclin E/Cdk2 and cyclin B/Cdk1 corresponding to conditions A to F in panel A are shown in Fig. S2. Other parameter values are as in Table S5.

Figure 3. Let-7 is a central component between the proliferation and a malignant transformation network. (A) Effect of the inflammatory circuit on the Cdk network dynamics. (A-D) Starting from a non-transformed, quiescent, cell state, defined by high Let-7 and low cyclin B/Cdk1 levels, a transient increase in Src from $t=100\text{h}$ to $t=105\text{h}$ (grey area) triggers a stable epigenetic switch and cell proliferation characterized by Let-7 down-regulation and sustained oscillations of cyclin B/Cdk1. From that transformed, cell proliferative state, the temporal evolution of Let-7 and cyclin B/Cdk1 are illustrated in the presence of transient (B) Lin28 inhibition (V_{SLIN28} passes from 0.1 to 0 for $500\text{h} < t < 1000\text{h}$), (C) NF- κ B inhibition ($k_{\text{AA1NFKB}} = k_{\text{AA2NFKB}} =$

$k_{AA3NFKB} = 0$ for $500h < t < 1000h$), or (D) PTEN over-expression ($V_{SMP_{PTEN}}$ passes from 0.001 to 10 for $500h < t < 1000h$). Other parameter values are as in Table S5.

Figure 4. Overexpression of cyclins is predicted to impact on the dynamics of the transformation network. Temporal evolution of (A) Let-7, or (B, C) Let-7 and cyclin B/Cdk1 illustrated for different synthesis rates of cyclins, V_{SMCYC} . From a non-transformed and quiescent cell state defined by high Let-7 levels and low levels of cyclin B/Cdk1, a sufficient increase in V_{SMCYC} (for $t > 100h$) down-regulates Let-7, allowing a stable switch to a transformed and cell proliferative state. Parameter values are as in Table S5.

Figure 5. Concomitant activation of the proliferation and transformation networks is cancer type-specific. (A) Cell proliferation index, CPI , and (B) Non-transformed state index, $NSTI$, calculated from the measured mRNA expression levels of the network components in the non-tumor, normal (N) samples, (green bars) and tumor condition (T, red bars) of 12 tumor cohorts from TCGA. In each case, mRNA levels are relative to the non-tumor condition. A list of all components used in the analysis can be found in Table S7.

Figure 6. Activation of the networks in tumors is heterogeneous among patients. (A) PCA analysis based on the expression of all network components in the non-tumor (blue dots) and tumor condition (red dots) of (A) cholangiocarcinoma, (B) hepatocellular carcinoma, and (C) prostate adenocarcinoma cohort from TCGA.

Figure 7. Modeling malignant cell transformation and cell proliferation distributions in a heterogeneous cell population. CPI versus $NSTI$ is shown in a heterogeneous cell population starting from a non-transformed, quiescent, cell state (A, C, E) or from a transformed, proliferative, cell state (B, D, F). In both states, 10% (A, B), 25% (C, D), or 50% (E, F) of uniform random variations from the basal value of each parameter are considered. Each circle corresponds to one cell in a population of 500 cells. Horizontal lines define an arbitrary threshold value for CPI , which is equal to 1, above which cells are considered into proliferation; vertical lines define an arbitrary threshold value for $NSTI$, equal to 10, above which cells are considered in a non-transformed state. Orange (A, C, E) and green dots (B, D, F)

correspond to the value of both indexes in the absence of random variation on parameters. Initial conditions are given in Table S6. Basal parameter values are as in Table S5.

Figure 8. A cell population model assesses the qualitative dynamics of the network switches in cholangiocarcinoma. mRNA levels of (A, B) cyclin B1 *versus* cyclin E1, (C, D) cyclin B1 *versus* Let-7c, and (E, F) Kras *versus* Let-7c of cholangiocarcinoma cohort from TCGA (A, C, E), and (B, D, F) in a model for a heterogeneous cell population where 50% of uniform random variations are considered around the basal value of each parameter. (B, D, F) Simulations are performed with a population of 100 cells. Initial conditions are given in Table S6. Src = 0.0000001 and other parameter values are as in Table S5. (A, C, E) green dots: non-tumor samples (n = 9), red dots: tumor samples (n = 36).

Figures

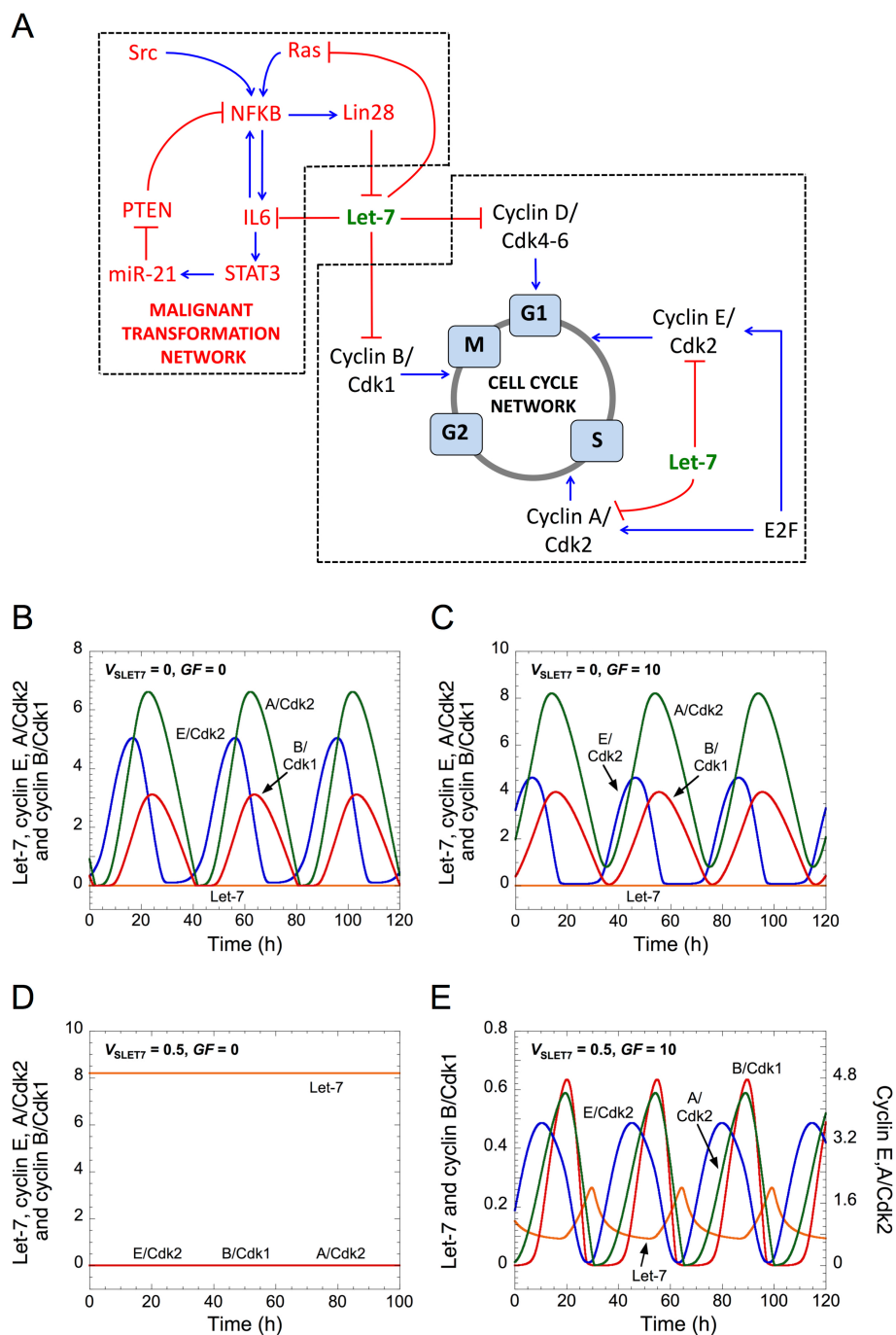


Figure 1

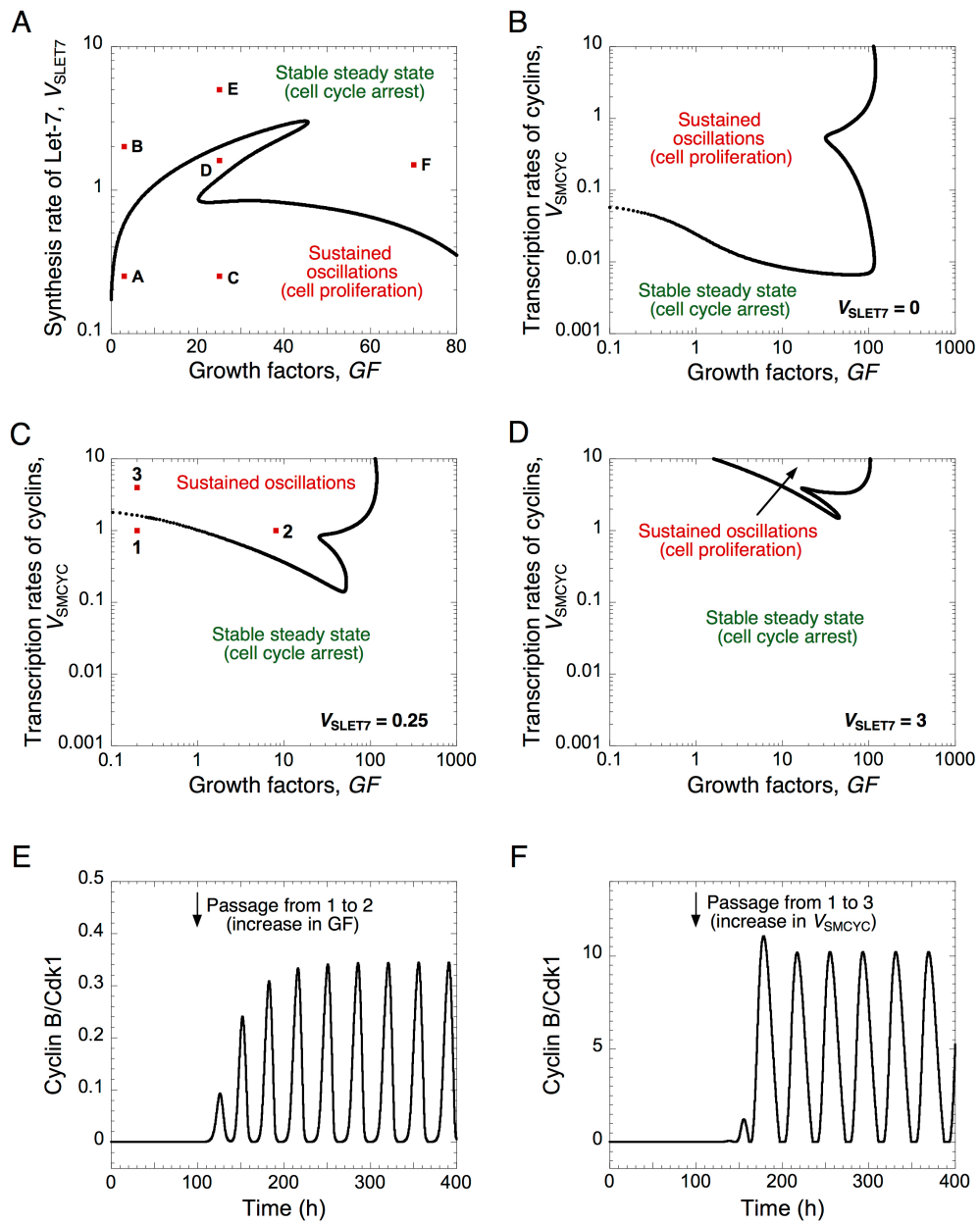


Figure 2

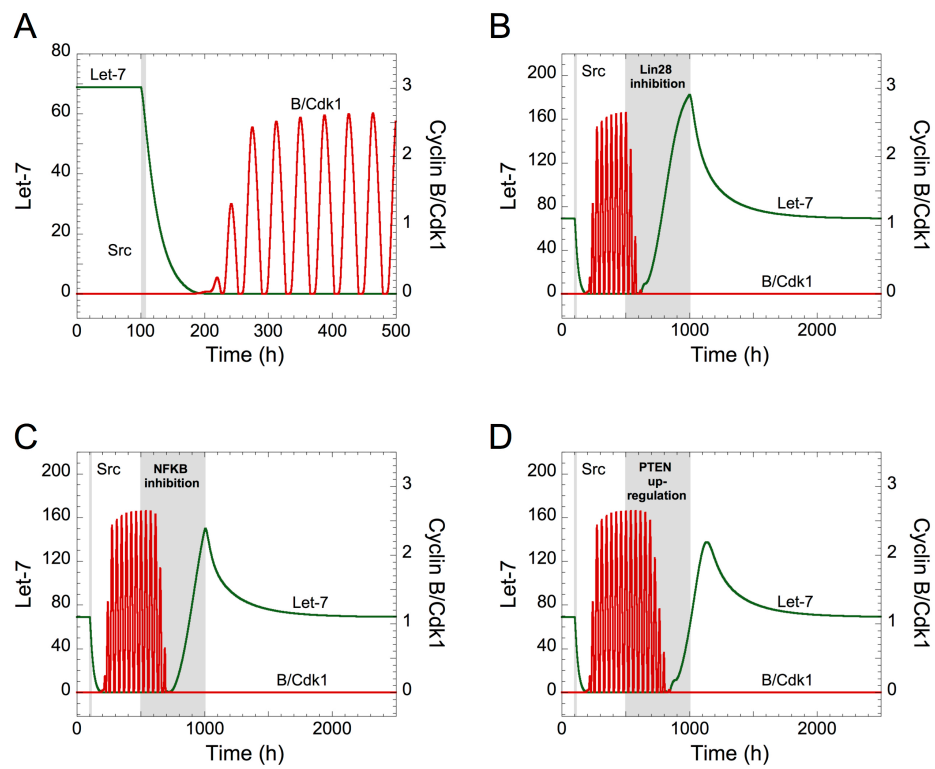


Figure 3

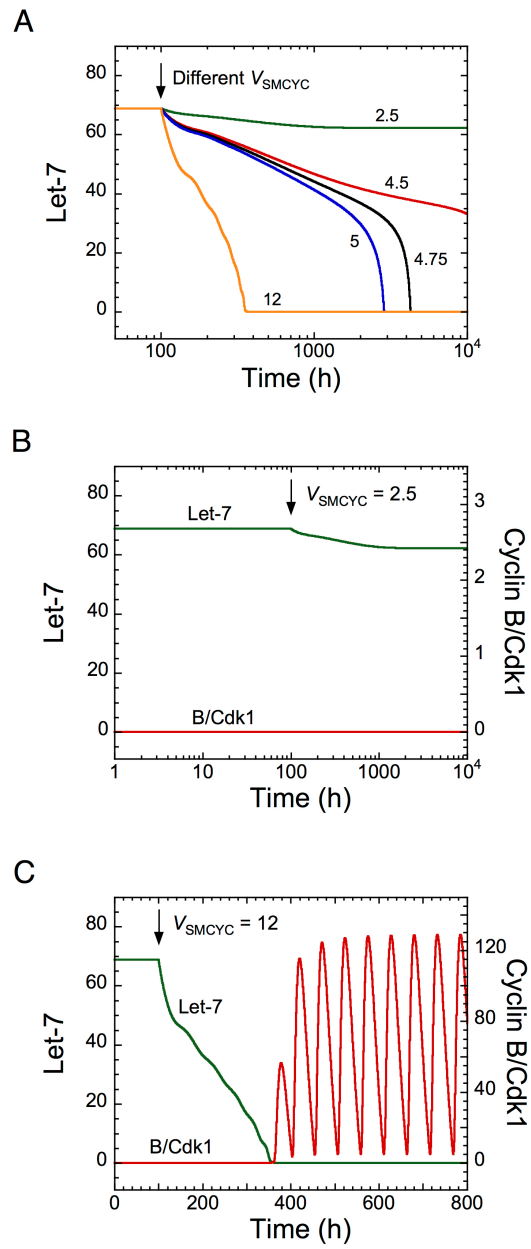


Figure 4

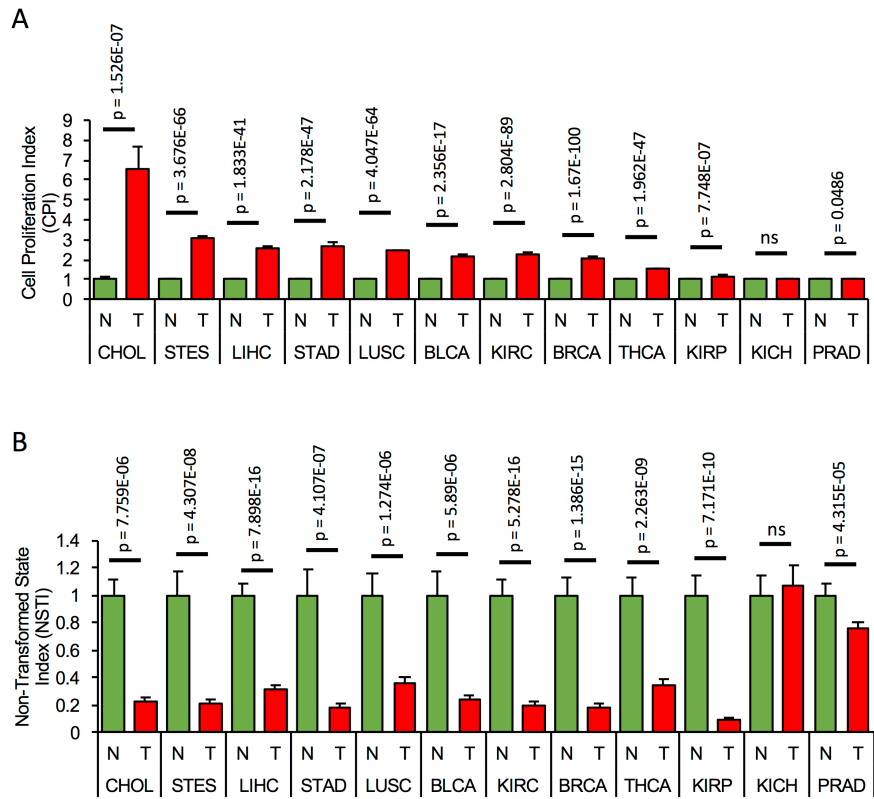


Figure 5

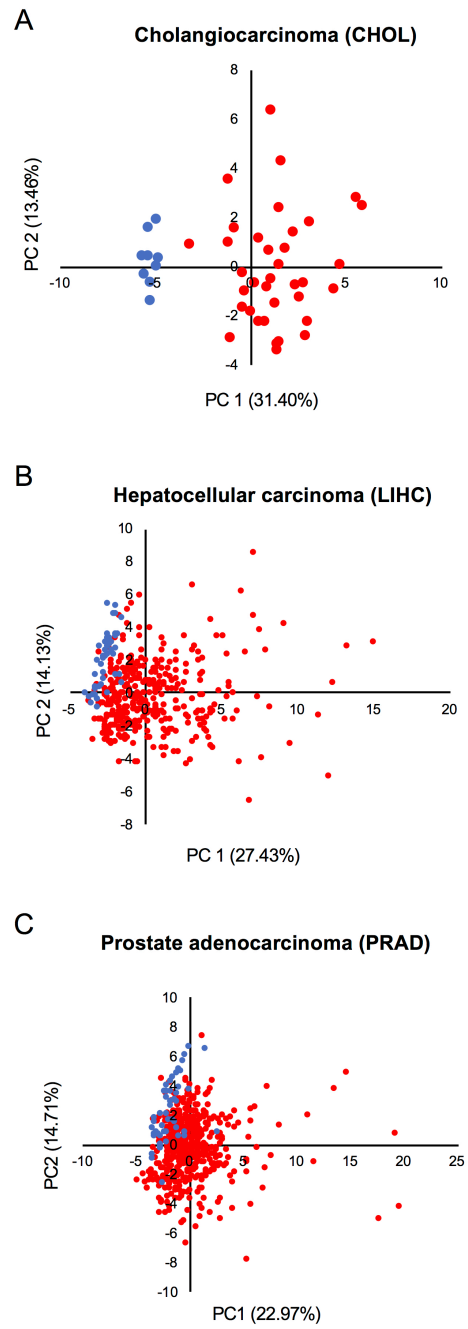


Figure 6

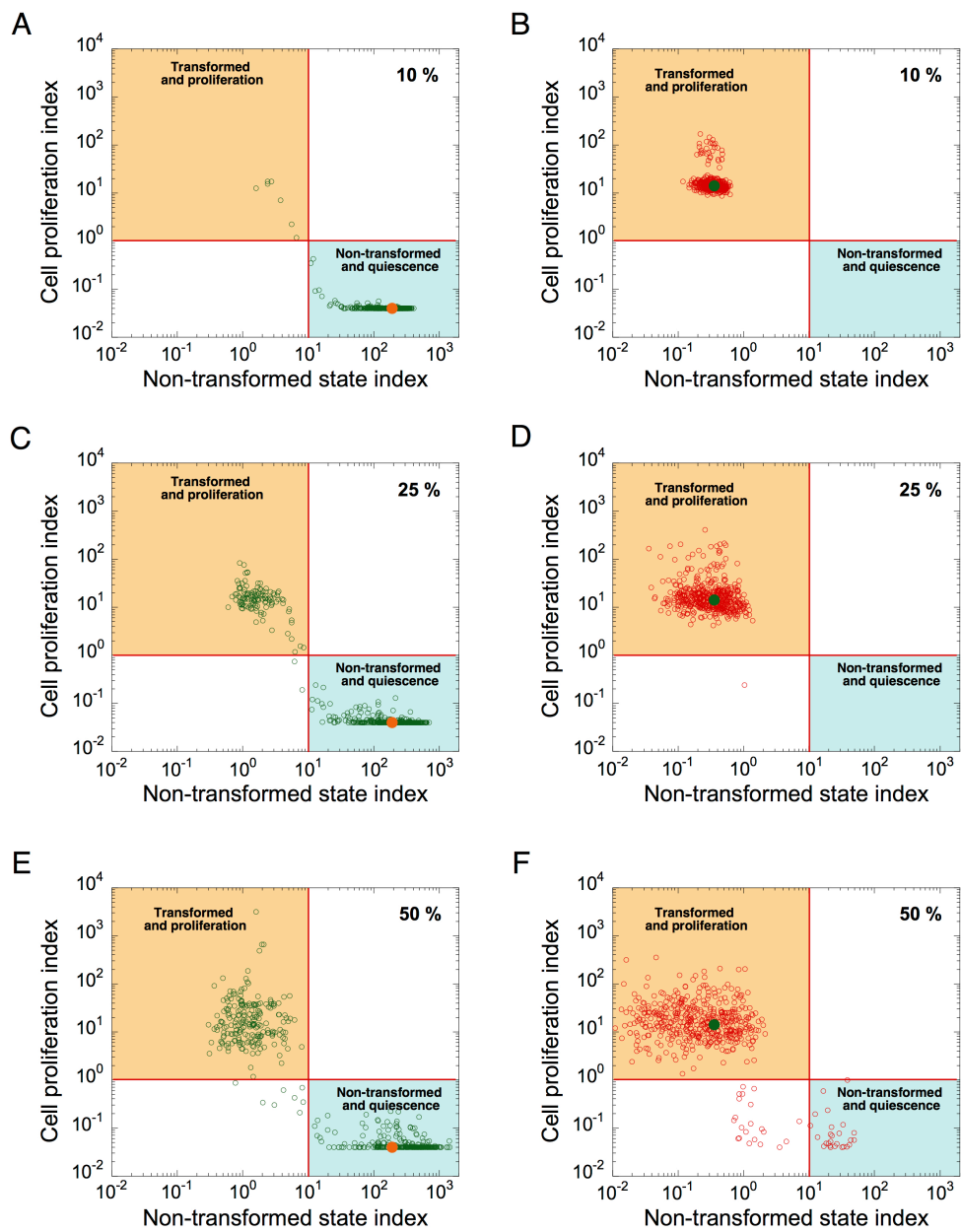


Figure 7

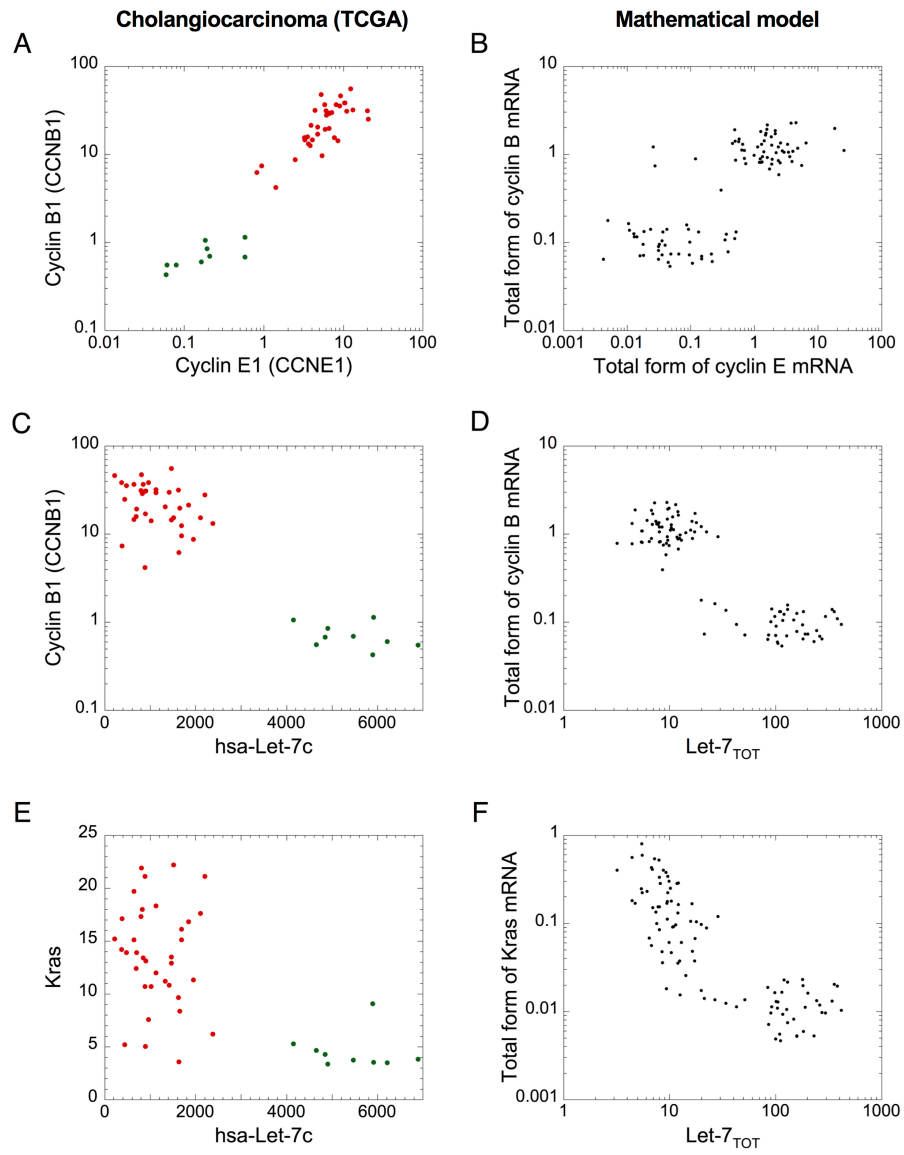


Figure 8

Supplementary Information

Modeling the dynamics of Let-7-coupled gene regulatory networks linking cell proliferation to malignant transformation

Claude Gérard¹, Frédéric Lemaigre¹ and Didier Gonze²

¹de Duve Institute, Université catholique de Louvain, Brussels, Belgium

²Unité de Chronobiologie théorique, Faculté des Sciences,
Université libre de Bruxelles, Brussels, Belgium

Table of contents

Description of the mathematical model	2
Numerical simulations and PCA analysis	2
Analysis of RNASeq and proteomic data	2
Sensitivity analysis of the cell cycle and cell transformation networks	3
Supplementary tables	4
Table S1. Variables of the mathematical model	4
Table S2. Predicted <i>CYCLIN</i> , <i>CDK</i> and <i>E2F</i> mRNAs targets of human Let-7 miRNA ..	5
Table S3. Kinetic equations of the mathematical model	6
Table S4. Detailed kinetic reactions of the mathematical model	9
Table S5. Parameters of the model	12
Table S6. Initial conditions	15
Table S7. List of the components used in the human tumors analysis (Figs. 5-6)	16
Supplementary figures	17
Figure S1. Detailed scheme of the model	17
Figure S2	18
Figure S3. Effect of the Cdk network on the transformation network dynamics	19
Figure S4. Sensitivity analysis	20
Figure S5. Growth factors couple transformation and proliferation networks in a Let-7-independent manner.	21
Figure S6. CPI correlates with cell cycle activation	22
Figure S7. Oncogenes and tumor suppressors control the robustness of the networks dynamics	23
Supplementary references	24

Description of the mathematical model

The model is composed of set of 15 kinetic equations governing the time dynamics of the Cdk network and 14 kinetics equations governing the dynamics of the inflammatory circuit which controls malignant transformation. The different variables of the model are defined in Table S1, the kinetics equations are found in Table S2, while the description of the parameters and their numerical values used in the simulations are in Table S5.

In the model, we consider for simplicity a constant total concentration of NF- κ B ($NFKB_{TOT}$), E2F ($E2F_{TOT}$), and APC (APC_{TOT}). Activation and inactivation reactions of E2F in Eq. [5], APC in Eq. [15] and NF- κ B in Eq. [16] behave as Goldbeter-Koshland switches (Goldbeter and Koshland, 1981). All other processes of the model rest on mass-action kinetics. Moreover, to couple the model for the Cdk network with the model for the inflammatory circuit leading to malignant transformation, equations [1] and [18] have been replaced by equation [18'].

Numerical simulations and PCA analysis

Numerical simulations were performed with XPPAUTO (<http://www.math.pitt.edu/~bard/xpp/xpp.html>) and matlab. The model is available on GitHub at the following address: <https://github.com/dgonze/Cell-cycle>.

Pearson correlation analysis were performed with rcorr function (Hmisc package), while PCA analysis were done with FactoMineR (Le, 2008).

Analysis of RNASeq and proteomic data

RNASeq and miRNASeq data of the different patient cohorts were retrieved from TCGA database (<http://firebrowse.org/>). For each cohort, we converted the “scaled_estimate” in the “illuminahisec_rnaseqv2_unc_edu_Level_3_RSEM_genes” file into TPM by multiplying by 10^6 .

Proteomic data from different cohorts in TCGA originate from The Cancer Proteome Atlas (<https://tcpaportal.org/tcpa/index.html>), where we used the RPPA dataset with the level 4 of data normalization.

RNASeq data of the 20 HCC cell lines arise from the OASIS Genomics portal (<http://www.oasis-genomics.org/>).

Sensitivity analysis of the cell cycle and cell transformation networks

Qualitative dynamics of the GRN, such as sustained oscillatory regime, stable steady states and bistable switches, critically depend on the GRN structure and the presence of gene regulatory motifs, i.e. positive and/or negative feedback loops (Tyson et al., 2003), but they may be also sensitive to kinetic parameter values. To assess the robustness of the behaviour with respect to parameter values, we performed a sensitivity analysis to identify which parameters of the mathematical model mostly influence the network dynamics. We increased or decreased by 20% the value of each parameter of the cell cycle network (Fig. S4A, B) and of the cell transformation network (Fig. S4C). For the cell cycle network, we plotted the change in amplitude and the change in period of the sustained oscillations of Cyclin B/Cdk1. The analysis indicated that the rates of synthesis of the cyclin/Cdk complexes, especially cyclin B/Cdk1, cyclin E/Cdk2 and cyclin A/Cdk2 are important parameters to control the amplitude and the period of the cyclin/Cdk oscillations. For the cell transformation network, we reported the change in the stable steady state level of Let-7 and we noted that the parameters driving the synthesis and degradation of Let-7 and LIN28 are important to control the levels of Let-7, and may induce a switch in the network if they are altered.

Supplementary tables

Table S1. Variables of the mathematical model

<u>Name</u>	<u>Definition</u>
<i>Model for the Cdk network, regulated by Let-7, driving the mammalian cell cycle</i>	
Let7	MicroRNA family Let-7
mMd	Cyclin D mRNA
Md	Active form of cyclin D/Cdk-4-6 complex
mMdlet7	Inactive complex between cyclin D mRNA (mMd) and Let7
E2F	Active form of the transcription factor E2F
mMe	Cyclin E mRNA
Me	Active form of cyclin E/Cdk2 complex
mMelet7	Inactive complex between cyclin E mRNA (mMe) and Let7
mMa	Cyclin A mRNA
Ma	Active form of cyclin A/Cdk2 complex
mMalet7	Inactive complex between cyclin A mRNA (mMa) and Let7
mMb	Cyclin B mRNA
Mb	Active form of cyclin B/Cdk1 complex
mMblet7	Inactive complex between cyclin B mRNA (mba) and Let7
APC	Anaphase-promoting complex regulating cyclin degradation
<i>Model for the inflammatory circuit driving the epigenetic switch to cell transformation</i>	
Let7	MicroRNA family Let-7
NFKB	Transcription factor NF- κ B
Lin28	RNA binding protein Lin28
mIL6	IL6 mRNA
mIL6Let7	Complex form between mIL6 and Let7
IL6	Interleukin 6 protein, which promotes the epigenetic switch leading to cell transformation
mRas	Ras oncogene mRNA
mRasLet7	Complex form between mRas and Let7
Ras	Protein form of Ras oncogene
STAT3	Transcription factor STAT3, whose activity is crucial to promote cell transformation
miR21	MicroRNA miR21, which represses the translation of the tumor suppressor PTEN
mPTEN	PTEN mRNA
miRmpten	Complex form between miR21 and mPTEN
PTEN	Protein form of the tumor suppressor PTEN (phosphatase and tensin homologue)

Table S2. Predicted *CYCLIN*, *CDK* and *E2F* mRNAs targets of human Let-7 miRNA

miRNA	mRNA targets with their (score)*
hsa-let-7a-2-3p	CCNB1 (70), E2F5 (54), E2F3 (79)
hsa-let-7a-3p	CCNA2 (98), CCNE2 (92), CDK6 (71), E2F5 (90), E2F6 (73), E2F7 (76), E2F8 (92)
hsa-let-7a-5p	CCND1 (65), CCND2 (98), E2F2 (83), E2F5 (94), E2F6 (91)
hsa-let-7b-3p	CDK6 (71), CCNA2 (98), CCNE2 (92), E2F8 (92), E2F5 (90), E2F7 (76), E2F6 (73)
hsa-let-7b-5p	E2F5 (94), E2F6 (91), E2F2 (83), CCND2 (98), CCND1 (65)
hsa-let-7c-3p	CCNB2 (72), E2F4 (92), E2F3 (91)
hsa-let-7c-5p	E2F2 (83), E2F5 (94), E2F6 (91), CCND1 (65), CCND2 (98)
hsa-let-7d-5p	E2F5 (94), E2F6 (90), E2F2 (83), CCND1 (56), CCND2 (98)
hsa-let-7e-5p	E2F5 (94), E2F6 (91), E2F2 (83), CCND2 (98), CCND1 (65)
hsa-let-7f-1-3p	CCNA2 (98), CCNE2 (92), CDK6 (71), E2F8 (92), E2F5 (90), E2F7 (76), E2F6 (73)
hsa-let-7f-2-3p	E2F6 (92), E2F5 (55), E2F8 (51)
hsa-let-7f-5p	CCND1 (65), CCND2 (98), E2F5 (94), E2F6 (91), E2F2 (83)

*The analysis was performed with miRDB, an online database for miRNA target prediction (<http://mirdb.org/>). Note that E2F family members can also be targeted by Let-7. In the mathematical model, to avoid an increase of the number of kinetic equations, we do not explicitly consider these regulations. Since E2F is a transcription factor that promote cyclin synthesis, a Let-7-mediated reduction of E2F protein level will reduce the synthesis of the different cyclins. Dynamically, this will be similar to a direct inhibition of the production of cyclin/Cdk complexes though binding of the cyclin to Let-7.

Table S3. Kinetic equations of the mathematical model

<i>Model for the Cdk network, regulated by Let-7, driving the mammalian cell cycle</i>	
$\frac{dLet7}{dt} = V_{SLET7} - k_1 \cdot mMd \cdot Let7 + k_2 \cdot mMdLet7 - k_3 \cdot mMe \cdot Let7 + k_4 \cdot mMeLet7 - k_5 \cdot mMa \cdot Let7 + k_6 \cdot mMaLet7 - k_7 \cdot mMb \cdot Let7 + k_8 \cdot mMbLet7 - k_{DLET7} \cdot Let7$	[1]
$\frac{dmMd}{dt} = V_{SMCYC} \cdot k_{SMMD} \cdot GF - k_1 \cdot mMd \cdot Let7 + k_2 \cdot mMdLet7 - k_{DMMD} \cdot mMd$	[2]
$\frac{dMd}{dt} = k_{SD} \cdot mMd - k_{DMD} \cdot Md$	[3]
$\frac{dmMdLet7}{dt} = k_1 \cdot mMd \cdot Let7 - k_2 \cdot mMdLet7 - k_{DMMDLET7} \cdot mMdLet7$	[4]
$\frac{dE2F}{dt} = k_{1E2F} \cdot \left(\frac{(E2F_{TOT} - E2F)}{K_{AE2F} + (E2F_{TOT} - E2F)} \right) \cdot (Md + Me) - k_{2E2F} \cdot \left(\frac{E2F}{K_{IE2F} + E2F} \right) \cdot Ma$	[5]
$\frac{dmMe}{dt} = V_{SMCYC} \cdot k_{SMME} \cdot E2F - k_3 \cdot mMe \cdot Let7 + k_4 \cdot mMeLet7 - k_{DMME} \cdot mMe$	[6]
$\frac{dMe}{dt} = k_{SE} \cdot mMe - k_{DME} \cdot Ma \cdot \left(\frac{Me}{K_{DE} + Me} \right) - k_{DME2} \cdot Me$	[7]
$\frac{dmMeLet7}{dt} = k_3 \cdot mMe \cdot Let7 - k_4 \cdot mMeLet7 - k_{DMMELET7} \cdot mMeLet7$	[8]
$\frac{dmMa}{dt} = V_{SMCYC} \cdot k_{SMMA} \cdot E2F - k_5 \cdot mMa \cdot Let7 + k_6 \cdot mMaLet7 - k_{DMMA} \cdot mMa$	[9]
$\frac{dMa}{dt} = k_{SA} \cdot mMa - k_{DMA} \cdot APC \cdot \left(\frac{Ma}{K_{DA} + Ma} \right) - k_{DMA2} \cdot Ma$	[10]
$\frac{dmMaLet7}{dt} = k_5 \cdot mMa \cdot Let7 - k_6 \cdot mMaLet7 - k_{DMMALET7} \cdot mMaLet7$	[11]
$\frac{dmMb}{dt} = V_{SMCYC} \cdot V_{SMMB} - k_7 \cdot mMb \cdot Let7 + k_8 \cdot mMbLet7 - k_{DMMB} \cdot mMb$	[12]
$\frac{dMb}{dt} = k_{SB} \cdot mMb \cdot Ma - k_{DMB} \cdot APC \cdot \frac{Mb}{K_{DB} + Mb} - k_{DMB2} \cdot Mb$	[13]

$$\frac{dmMbLet7}{dt} = k_7 \cdot mMb \cdot Let7 - k_8 \cdot mMbLet7 - k_{DMMBLET7} \cdot mMbLet7 \quad [14]$$

$$\frac{dAPC}{dt} = k_{1APC} \cdot \left(\frac{APC_{TOT} - APC}{K_{AAPC} + (APC_{TOT} - APC)} \right) \cdot Mb - V_{2APC} \cdot \left(\frac{APC}{K_{1APC} + APC} \right) \quad [15]$$

Model for the inflammatory circuit driving the epigenetic switch to cell transformation

$$\begin{aligned} \frac{dNFKB}{dt} = & (k_{AA1NFKB} \cdot Src + k_{AA2NFKB} \cdot IL6 + k_{AA3NFKB} \cdot Ras) \cdot \left(\frac{K_{IPTEN}}{K_{IPTEN} + PTEN} \right) \cdot \left(\frac{NFKB_1}{K_{ANFKB} + NFKB_1} \right) \\ & - V_{DNFKB} \cdot \left(\frac{NFKB}{K_{INFKB} + NFKB} \right) \end{aligned} \quad [16]$$

$$\frac{dLIN28}{dt} = V_{SLIN28} \cdot \left(\frac{NFKB}{K_{AINFKB} + NFKB} \right) - k_{DLIN28} \cdot LIN28 \quad [17]$$

$$\begin{aligned} \frac{dLet7}{dt} = & V_{SLET7} \cdot \left(\frac{K_{ILET7}}{K_{ILET7} + LIN28} \right) - k_9 \cdot mL6 \cdot Let7 + k_{10} \cdot mL6Let7 - k_{11} \cdot mRas \cdot Let7 + k_{12} \cdot mRasLet7 \\ & - k_{DLET7} \cdot Let7 \end{aligned} \quad [18]$$

$$\frac{dmIL6}{dt} = V_{S1MIL6} + V_{S2MIL6} \cdot \left(\frac{NFKB}{K_{A2NFKB} + NFKB} \right) - k_9 \cdot mL6 \cdot Let7 + k_{10} \cdot mL6Let7 - k_{DMIL6} \cdot mL6 \quad [19]$$

$$\frac{dmIL6Let7}{dt} = k_9 \cdot mL6 \cdot Let7 - k_{10} \cdot mL6Let7 - k_{DILLET} \cdot mL6Let7 \quad [20]$$

$$\frac{dIL6}{dt} = k_{SIL6} \cdot mL6 - k_{DIL6} \cdot IL6 \quad [21]$$

$$\frac{dmRas}{dt} = V_{SMRAS} - k_{11} \cdot mRas \cdot Let7 + k_{12} \cdot mRasLet7 - k_{DMRAS} \cdot mRas \quad [22]$$

$$\frac{dmRasLet7}{dt} = k_{11} \cdot mRas \cdot Let7 - k_{12} \cdot mRasLet7 - k_{DRASLET} \cdot mRasLet7 \quad [23]$$

$$\frac{dRas}{dt} = k_{SRAS} \cdot mRas - k_{DRAS} \cdot Ras \quad [24]$$

$$\frac{dSTAT3}{dt} = V_{SSTAT} \cdot \left(\frac{IL6}{K_{A2IL6} + IL6} \right) - k_{DSTAT} \cdot STAT3 \quad [25]$$

$\frac{dmiR21}{dt} = V_{SMIR21} \cdot \left(\frac{STAT3}{K_{ASTAT} + STAT3} \right) - k_{13} \cdot miR21 \cdot mPTEN + k_{14} \cdot miRmPTEN - k_{DMIR21} \cdot miR21$	[26]
$\frac{dmPTEN}{dt} = V_{SMPTEN} - k_{13} \cdot miR21 \cdot mPTEN + k_{14} \cdot miRmPTEN - k_{DMPTEN} \cdot mPTEN$	[27]
$\frac{dPTEN}{dt} = k_{SPTEN} \cdot mPTEN - k_{DPTEN} \cdot PTEN$	[28]
$\frac{dmiRmPTEN}{dt} = k_{13} \cdot miR21 \cdot mPTEN - k_{14} \cdot miRmPTEN - k_{DMIRMP} \cdot miRmPTEN$	[29]
<i>Coupling the Cdk network and the epigenetic switch through Let-7 miRNA</i>	
$\begin{aligned} \frac{dLet7}{dt} = & V_{SLET7} \cdot \left(\frac{K_{ILET7}}{K_{ILET7} + LIN28} \right) - k_9 \cdot mL6 \cdot Let7 + k_{10} \cdot mL6Let7 - k_{11} \cdot mRas \cdot Let7 + k_{12} \cdot mRasLet7 \\ & - k_1 \cdot mMd \cdot Let7 + k_2 \cdot mMdLet7 - k_3 \cdot mMe \cdot Let7 + k_4 \cdot mMeLet7 - k_5 \cdot mMa \cdot Let7 + k_6 \cdot mMaLet7 \\ & - k_7 \cdot mMb \cdot Let7 + k_8 \cdot mMbLet7 - k_{DLET7} \cdot Let7 \end{aligned}$	[18']
<i>Simulations where GF promote cyclin D synthesis and activate Ras</i>	
$\frac{dRas}{dt} = k_{S1RAS} \cdot mRas + k_{S2RAS} \cdot GF - k_{DRAS} \cdot Ras$	[24']

Where:

$$NFKB_{TOT} = NFKB + NFKB_I$$

Table S4. Detailed kinetic reactions of the mathematical model

Reaction number (Fig. S1)	Definition of the reaction	Reaction
1	Activation of NFkB by Src	$k_{AA1NFkB} \cdot Src \cdot \left(\frac{K_{IPTEN}}{K_{IPTEN} + PTEN} \right) \cdot \left(\frac{NFkB_I}{K_{ANFkB} + NFkB_I} \right)$
2	Activation of NFkB by Ras	$k_{AA3NFkB} \cdot Ras \cdot \left(\frac{K_{IPTEN}}{K_{IPTEN} + PTEN} \right) \cdot \left(\frac{NFkB_I}{K_{ANFkB} + NFkB_I} \right)$
3	Activation of LIN28 synthesis by NFkB	$V_{SLIN28} \cdot \left(\frac{NFkB}{K_{A1NFkB} + NFkB} \right)$
4	Inhibition of Let-7 by LIN28	$V_{SLET7} \cdot \left(\frac{K_{ILET7}}{K_{ILET7} + LIN28} \right)$
5	Association/dissociation of Let-7 and mL6 (inhibition of mL6 translation by Let-7)	$- k_9 \cdot mL6 \cdot Let7 + k_{10} \cdot mL6Let7$
6	Association/dissociation of Let-7 and mRas (inhibition of mRas translation by Let-7)	$- k_{11} \cdot mRas \cdot Let7 + k_{12} \cdot mRasLet7$
7	Activation of NFkB by IL6	$k_{AA2NFkB} \cdot IL6 \cdot \left(\frac{K_{IPTEN}}{K_{IPTEN} + PTEN} \right) \cdot \left(\frac{NFkB_I}{K_{ANFkB} + NFkB_I} \right)$
8	Activation of mL6 transcription by NFkB	$V_{S2MIL6} \cdot \left(\frac{NFkB}{K_{A2NFkB} + NFkB} \right)$
9	Activation of STAT3 synthesis by IL6	$k_{SSTAT} \cdot \left(\frac{IL6}{K_{A2IL6} + IL6} \right)$
10	Activation of miR-21 synthesis by STAT3	$k_{SMIR21} \cdot \left(\frac{STAT3}{K_{ASTAT} + STAT3} \right)$
11	Association/dissociation of miR-21 and mPTEN (inhibition of mPTEN translation by miR-21)	$- k_{13} \cdot miR21 \cdot mPTEN + k_{14} \cdot miRmPTEN$
12	Inhibition of NFkB by PTEN	See reactions 1, 2 and 7
13	Association/dissociation of Let-7 and mMd (inhibition of mMd translation by Let-7)	$- k_1 \cdot mMd \cdot Let7 + k_2 \cdot mMdLet7$
14	Association/dissociation of Let-7 and mMe (inhibition of mMe translation by Let-7)	$- k_3 \cdot mMe \cdot Let7 + k_4 \cdot mMeLet7$
15	Association/dissociation of Let-7 and mMa (inhibition of mMa translation by Let-7)	$- k_5 \cdot mMa \cdot Let7 + k_6 \cdot mMaLet7$
16	Association/dissociation of Let-7 and mMb (inhibition of mMb translation by Let-7)	$- k_7 \cdot mMb \cdot Let7 + k_8 \cdot mMbLet7$
17	Activation of mMd transcription by GF	$V_{SMYC} \cdot k_{SMMD} \cdot GF$
18	Transcription rate of mMd	See reaction 17
18	Translation of Md	$k_{SD} \cdot mMd$
19	Degradation of mMd	$- k_{DMMD} \cdot mMd$
19	Degradation of Md	$- k_{DMd} \cdot Md$
20	Activation of E2F by Md	$k_{1E2F} \cdot \left(\frac{E2F_{TOT} - E2F}{K_{AE2F} + E2F_{TOT} - E2F} \right) \cdot Md$
21	Activation of E2F by Me	$k_{1E2F} \cdot \left(\frac{E2F_{TOT} - E2F}{K_{AE2F} + E2F_{TOT} - E2F} \right) \cdot Me$
22	Activation of mMe transcription by E2F	$V_{SMYC} \cdot k_{SMME} \cdot E2F$
22	Translation of Me	$k_{SE} \cdot mMe$

23	Degradation of mMe	$-k_{DMME} \cdot mMe$
23	Degradation of Me	$-k_{DME2} \cdot Me$
24	Activation of Me degradation by Ma	$k_{DME} \cdot Ma \cdot \left(\frac{Me}{K_{DE} + Me} \right)$
25	Activation of mMe transcription by E2F	See Reaction 22
26	Activation of mM transcription by E2F	$V_{SMYC} \cdot k_{SMA} \cdot E2F$
27	Inactivation of E2F by Ma	$-k_{2E2F} \cdot \left(\frac{E2F}{K_{IE2F} + E2F} \right) \cdot Ma$
28	Activation of E2F	See Reactions 20 and 21
29	Inactivation of E2F	See Reaction 27
30	mM transcription	See Reaction 26
30	Ma translation	$k_{SA} \cdot mM$
31	Degradation of mM	$-k_{DMA} \cdot mM$
32	Degradation of Ma	$-k_{DMA2} \cdot Ma$
33	Transcription of mMb	$V_{SMCYC} \cdot V_{SMB}$
33	Activation of Mb synthesis by Ma	$k_{SB} \cdot mMb \cdot Ma$
34	Degradation of mMb	$-k_{DMMB} \cdot mMb$
34	Degradation of Mb	$-k_{DMB2} \cdot Mb$
35	Activation of APC promoted by Mb	$k_{1APC} \cdot \left(\frac{APC_{TOT} - APC}{K_{AAPC} + APC_{TOT} - APC} \right) \cdot Mb$
36	Activation of APC	See Reaction 35
37	Inactivation of APC	$V_{2APC} \cdot \left(\frac{APC}{K_{IAPC} + APC} \right)$
38	Degradation of Mb activated by APC	$-k_{DMB} \cdot APC \cdot \left(\frac{Mb}{K_{DB} + Mb} \right)$
39	Degradation of Ma activated by APC	$-k_{DMA} \cdot APC \cdot \left(\frac{Ma}{K_{DA} + Ma} \right)$
For sake of simplicity, the following reactions were not numbered in the wiring diagram of Fig. S1		
	Synthesis rate of Let7	V_{SLET7}
	Degradation rate of Let7	$-k_{DLET7} \cdot Let7$
	Activation of LIN28 synthesis by NFkB	$V_{SLIN28} \cdot \left(\frac{NFkB}{K_{A1NFkB} + NFkB} \right)$
	Degradation of mMdLet7	$-k_{DMMDLET7} \cdot mMdLet7$
	Degradation of mMeLet7	$-k_{DMMELET7} \cdot mMeLet7$
	Degradation of mMALet7	$-k_{DMMALLET7} \cdot mMALet7$
	Degradation of mMBLet7	$-k_{DMMBLET7} \cdot mMBLet7$
	Inactivation of NFkB	$-V_{DNFkB} \cdot \left(\frac{NFkB}{K_{INFkB} + NFkB} \right)$
	Degradation of LIN28	$-k_{DLIN28} \cdot LIN28$
	Basal transcription rate of mIL6	V_{S1MIL6}
	Transcription of mIL6 activated by NFkB	$V_{S2MIL6} \cdot \left(\frac{NFkB}{K_{A2NFkB} + NFkB} \right)$
	Degradation of mIL6	$-k_{DMIL6} \cdot mIL6$
	Degradation of mIL6Let7	$-k_{DILLET7} \cdot mIL6Let7$
	Translation of IL6	$k_{SIL6} \cdot mIL6$

	Degradation of IL6	$-k_{DIL6} \cdot IL6$
	Transcription of mRas	V_{SMRAS}
	Degradation of mRas	$-k_{DMRAS} \cdot mRas$
	Degradation of mRasLet7	$-k_{DRASLET} \cdot mRasLet7$
	Translation of Ras	$k_{SRAS} \cdot mRas$
	Degradation of Ras	$-k_{DRAS} \cdot Ras$
	Synthesis of STAT3 activated by IL6	$V_{SSTAT} \cdot \left(\frac{IL6}{K_{A2IL6} + IL6} \right)$
	Degradation of STAT3	$-k_{DSTAT} \cdot STAT3$
	Synthesis of miR21 activated by STAT3	$V_{SMIR21} \cdot \left(\frac{STAT3}{K_{ASTAT} + STAT3} \right)$
	Degradation of miR21	$-k_{DMIR21} \cdot miR21$
	Transcription of mPTEN	V_{SMPTEN}
	Degradation of mPTEN	$-k_{DMPTEN} \cdot mPTEN$
	Translation of PTEN	$k_{SPTEN} \cdot mPTEN$
	Degradation of PTEN	$-k_{DPTEN} \cdot PTEN$
	Degradation of miRmPTEN	$-k_{DMIRMP} \cdot miRmPTEN$

Table S5. Parameters of the model

<u>Symbol</u>	<u>Definition</u>	<u>Numerical value</u>
<i>Model for the Cdk network, regulated by Let-7, driving the mammalian cell cycle</i>		
V_{SMCYC}	Multiplying factor for the rates of synthesis of all cyclins	1.5
GF	Growth factors	1
k_{SMMD}	Transcription rate constant of cyclin D	0.03
k_{DMMD}	Rate constant for the degradation of cyclin D messenger RNA	0.2
k_{SD}	Translation rate constant of cyclin D	0.5
k_{DMD}	Rate constant for the degradation of cyclin D protein	0.5
k_{1E2F}	Rate constant for activation of E2F	2.3
K_{AE2F}	Michaelis constant for E2F activation	0.01
k_{2E2F}	Rate constant for inactivation of E2F	2.5
K_{IE2F}	Michaelis constant for E2F inactivation	0.01
$E2F_{TOT}$	Total level of E2F transcription factor	4
k_{SMME}	Transcription rate constant of cyclin E	0.035
k_{DMME}	Rate constant for the degradation of cyclin E messenger RNA	0.08
k_{SE}	Translation rate constant of cyclin E	0.8
k_{DME}	Rate constant for the degradation of cyclin E protein regulated by cyclin A/Cdk2	0.2
k_{DME2}	Basal rate constant for the degradation of cyclin E protein	0.1
K_{DE}	Michaelis constant for degradation of cyclin E/Cdk2 by cyclin A/Cdk2	0.08
k_{SMMA}	Transcription rate constant of cyclin A	0.07
k_{DMMA}	Rate constant for the degradation of cyclin A messenger RNA	0.08
k_{SA}	Translation rate constant of cyclin A	0.4
k_{DMA}	Rate constant for the degradation of cyclin A protein regulated by APC	0.35
k_{DMA2}	Basal rate constant for the degradation of cyclin A protein	0.05
K_{DA}	Michaelis constant for degradation of cyclin A/Cdk2 by APC	0.01
V_{SMMB}	Transcription rate of cyclin B	0.06
k_{DMMB}	Rate constant for the degradation of cyclin B messenger RNA	0.08
k_{SB}	Translation rate constant of cyclin B	0.35
k_{DMB}	Rate constant for the degradation of cyclin B protein regulated by APC	0.2
k_{DMB2}	Basal rate constant for the degradation of cyclin B protein	0.7
K_{DB}	Michaelis constant for degradation of cyclin B/Cdk1 by APC	0.01
k_{1APC}	Rate constant for activation of APC through cyclin B/Cdk1	1
K_{AAPC}	Michaelis constant for APC activation	2
V_{2APC}	Inactivation rate of APC	0.3
K_{IAPC}	Michaelis constant for APC inactivation	10
APC_{TOT}	Total level of the Anaphase-Promoting Complex	2
k_1	Bimolecular rate constant for binding of Let7 to mMd	1
k_2	Rate constant for dissociation of complex (mMdlet7) between Let7 and mMd	0.01
k_3	Bimolecular rate constant for binding of Let7 to mMe	1
k_4	Rate constant for dissociation of complex (mMelet7) between Let7 and	0.01

	mMe	
k_5	Bimolecular rate constant for binding of Let7 to mMa	1
k_6	Rate constant for dissociation of complex (mMalet7) between Let7 and mMa	0.01
k_7	Bimolecular rate constant for binding of Let7 to mMb	1
k_8	Rate constant for dissociation of complex (mMblet7) between Let7 and mMb	0.01
k_{DMMDLET7}	Rate constant for degradation of mMdlet7 complex	1
k_{DMMELET7}	Rate constant for degradation of mMelet7 complex	1
k_{DMMALET7}	Rate constant for degradation of mMalet7 complex	1
k_{DMMBLET7}	Rate constant for degradation of mMblet7 complex	1
Model for the inflammatory circuit driving the epigenetic switch to cell transformation		
Src	Src kinase oncoprotein (signal triggering an inflammatory response leading to an activation of the epigenetic switch)	0
V_{SLET7}	Maximum rate of synthesis of Let7 microRNA	10
K_{ILET7}	Michaelis constant for the repression of Let7 synthesis by Lin28	0.1
k_{DLET7}	Rate constant for degradation of Let7 microRNA	0.05
k_{AA1NFKB}	Rate constant for the activation of NF- κ B by Src	10
k_{AA2NFKB}	Rate constant for the activation of NF- κ B by IL6	0.09
k_{AA3NFKB}	Rate constant for the activation of NF- κ B by Ras	1
K_{ANFKB}	Michaelis constant for the activation of NF- κ B	0.01
K_{INFKB}	Michaelis constant for the inhibition of NF- κ B	0.02
V_{DNFKB}	Maximum rate for NF- κ B inactivation	0.01
NFKB_{TOT}	Total concentration of NF- κ B	1
K_{A2IL6}	Michaelis constant for the activation of STAT3 synthesis by IL6	40
K_{IPTEN}	Michaelis constant for the inhibition of NF- κ B activation by PTEN	5
V_{SLIN28}	Maximum rate of synthesis of Lin28	0.1
k_{DLIN28}	Rate constant for the degradation of Lin28	0.015
K_{A1NFKB}	Michaelis constant for the activation of Lin28 synthesis by NF- κ B	0.01
k_9	Bimolecular rate constant for binding of Let7 to mL6	10
k_{10}	Rate constant for dissociation of complex (mL6let7) between Let7 and mL6	0.01
V_{S1MIL6}	Independent rate of synthesis of IL6 mRNA, mL6	0.12
V_{S2MIL6}	Maximum rate of synthesis of IL6 mRNA depending on NF- κ B	0.01
k_{DMIL6}	Rate constant for the degradation of IL6 mRNA	0.01
k_{DILLET}	Rate constant for the degradation of the complex between Let7 and mL6	0.5
K_{A2NFKB}	Michaelis constant for the activation of mL6 synthesis by NF- κ B	5
k_{SIL6}	Rate constant for the synthesis of IL6 protein	1.5
k_{DIL6}	Rate constant for the degradation of IL6 protein	0.4
V_{SMRAS}	Rate of synthesis of Ras mRNA, mRas	0.006
k_{11}	Bimolecular rate constant for binding of Let7 to mRas	10
k_{12}	Rate constant for dissociation of complex (mRaslet7) between Let7 and mRas	0.01
k_{DMRAS}	Rate constant for the degradation of mRas	0.01
k_{DRASLET}	Rate constant for the degradation of the complex between Let7 and mRas	0.5
k_{SRAS}	Rate constant for the synthesis of Ras protein	0.25

k_{DRAS}	Rate constant for the degradation of Ras protein	0.03
V_{SSTAT}	Maximum rate of synthesis of STAT3	0.15
k_{DSTAT}	Rate constant for the degradation of STAT3	0.03
V_{SMIR21}	Maximum rate of synthesis of miR21 microRNA	4
K_{ASTAT}	Michaelis constant for the activation miR21 synthesis by STAT3	5
k_{13}	Bimolecular rate constant for binding of miR21 to mPTEN	10
k_{14}	Rate constant for dissociation of complex (miRmpten) between miR21 and mPTEN	0.01
k_{DMIR21}	Rate constant for the degradation of miR21	0.2
V_{SMPTEN}	Rate of synthesis of PTEN mRNA, mPTEN	0.001
k_{DMPTEN}	Rate constant for the degradation of mPTEN	0.1
k_{DMIRMP}	Rate constant for the degradation of the complex between mPTEN and miR21	0.1
k_{SPTEN}	Rate constant for the synthesis of PTEN protein	0.9
k_{DPTEN}	Rate constant for the degradation of PTEN protein	0.05

Note: We focus our study on qualitative rather than quantitative aspects in the expression of the different components of the network. Indeed, our goal is to analyze the dynamic implications of the regulatory structure of the epigenetic switch linking inflammation to cell transformation and of the Cdk network driving the cell cycle, i.e. the wiring diagram, or the topology, which is crucial for the network behavior (see (Wagner, 2005)). Moreover, many parameters have not been determined experimentally. The numerical values have been selected to yield a non-transformed state without the inflammatory signal, Src; or a transformed state within less than 100h with transient inflammatory signal, which corresponds to experimental observations (Iliopoulos et al., 2009). For the Cdk network, parameter values have been chosen to reach cell cycle periods ranging between 20h and 50h, which correspond to the doubling time in most mammalian cells (Alexiades and Cepko, 1996). The time units for the parameters in the model are expressed in hours, while the concentrations are expressed in μM . In addition, the mechanisms controlling miRNA degradation are complex and not fully understood (Zhang et al., 2012). As a consequence, we assume that each complex between a mRNA and its regulating miRNA is rapidly targeted for degradation. The rate constants for the degradation of mRNAs and proteins are between 0.01h^{-1} and 1h^{-1} . This range of values corresponds to a half-life duration between 0.69h and 69.3h, which is in agreement with experimentally-measured half-life durations (Schwanhaeusser et al., 2011). Indeed, Schwanhäusser *et al.* showed that the mean half-life duration is 9h and 46h, for mRNAs and proteins, respectively. In the model, the short half-life durations of 0.69h are the half-life durations of the complexes between Let-7 and the mRNAs of the different cyclins. Such short half-life durations are in line with the fact that miRNA binding to its target mRNAs rapidly leads to its degradation (Wu et al., 2006).

Table S6. Initial conditions

Variable	C.I. # 1 (leads to non- transformed cell)	C.I. # 2 (leads to transformed cell)	C.I. # 3 (cell population, Figs. 8B,D,F)
<i>NFKB</i>	0.00045	0.5	0.01
<i>Lin28</i>	0.34	0.5	0.01
<i>Let7</i>	40	1	0
<i>mIL6</i>	0.0003	0.5	0.01
<i>IL6</i>	0.001	0.5	0.01
<i>mRas</i>	0.00001	0.5	0.01
<i>Ras</i>	0.0001	0.5	0.01
<i>STAT3</i>	0.0001	0.5	0.01
<i>mPTEN</i>	0.01	0	0.01
<i>mRasLet7</i>	0	0	0
<i>mIL6Let7</i>	0	0	0
<i>miR21</i>	0.0003	0	0
<i>PTEN</i>	0.17	0	0
<i>MiRmPTEN</i>	0	0	0

Table S7. List of the components used in the human tumors analysis (Figs. 5-6)

# of components	Symbol	Name
1	CCNA1	Cyclin A1
2	CCNB1	Cyclin B1
3	CCND1	Cyclin A1
4	CCNE1	Cyclin E1
5	CDK1	Cyclin-dependent kinase 1
6	CDK2	Cyclin-dependent kinase 2
7	CDK4	Cyclin-dependent kinase 4
8	CDK6	Cyclin-dependent kinase 6
9	E2F1	E2F transcription factor 1
10	E2F2	E2F transcription factor 2
11	E2F3	E2F transcription factor 3
12	E2F4	E2F transcription factor 4
13	E2F5	E2F transcription factor 5
14	E2F6	E2F transcription factor 6
15	E2F7	E2F transcription factor 7
16	E2F8	E2F transcription factor 8
17	IL6R	Interleukin 6 receptor
18	IL6	Interleukin 6
19	KRAS	KRAS oncogene
20	LIN28A	Lin-28 homolog A
21	LIN28B	Lin-28 homolog B
22	PTEN	Phosphatase and tensin homolog
23	STAT3	Signal transducer and activator of transcription 3
24	hsa-let-7a-1	MicroRNA let-7a-1
25	hsa-let-7a-2	MicroRNA let-7a-2
26	hsa-let-7a-3	MicroRNA let-7a-3
27	hsa-let-7b	MicroRNA let-7b
28	hsa-let-7c	MicroRNA let-7c
29	hsa-let-7d	MicroRNA let-7d
30	hsa-let-7e	MicroRNA let-7e
31	hsa-mir-21	MicroRNA 21

Supplementary figures

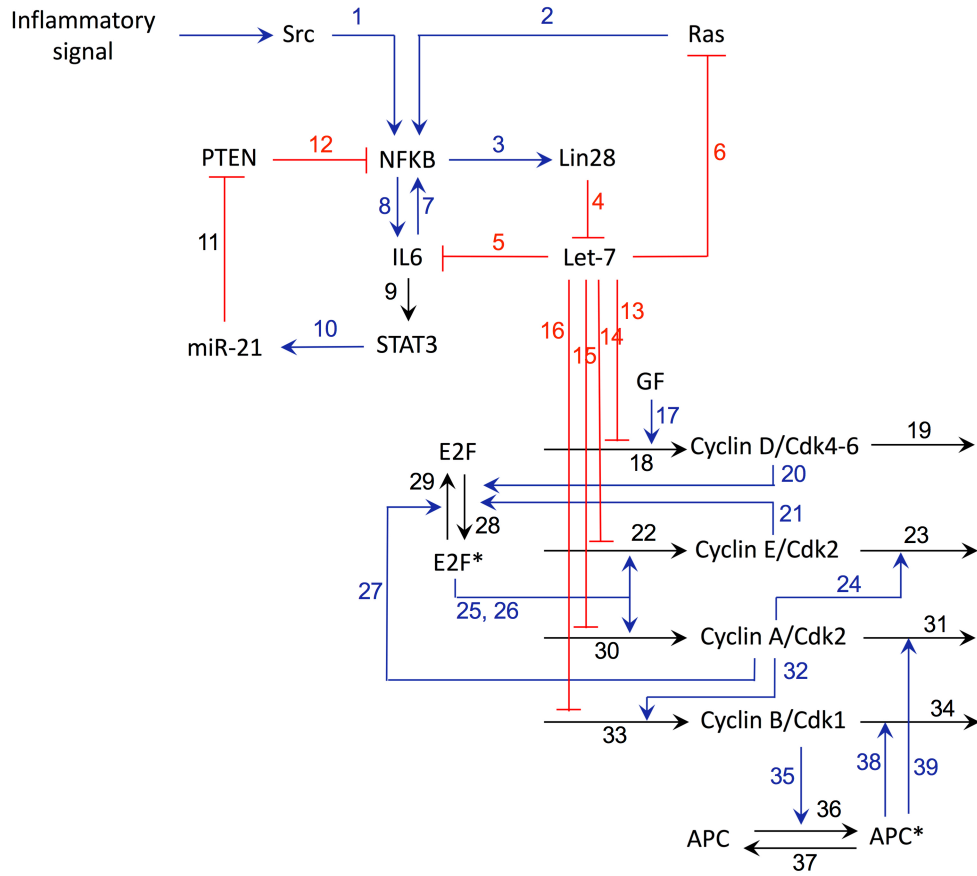


Figure S1. Detailed scheme of the model. The model for the epigenetic switch linking inflammation to cell transformation originates from a previous study (Gerard et al., 2014), while the model for the post-transcriptional regulation of the Cdk network by Let-7 is based on a skeleton model for the Cdk network driving the mammalian cell cycle (Gerard and Goldbeter, 2011). Let-7, which is involved in multiple positive feedback loops, can repress the synthesis of each cyclin. Numbers correspond to the equations given in Table S4.

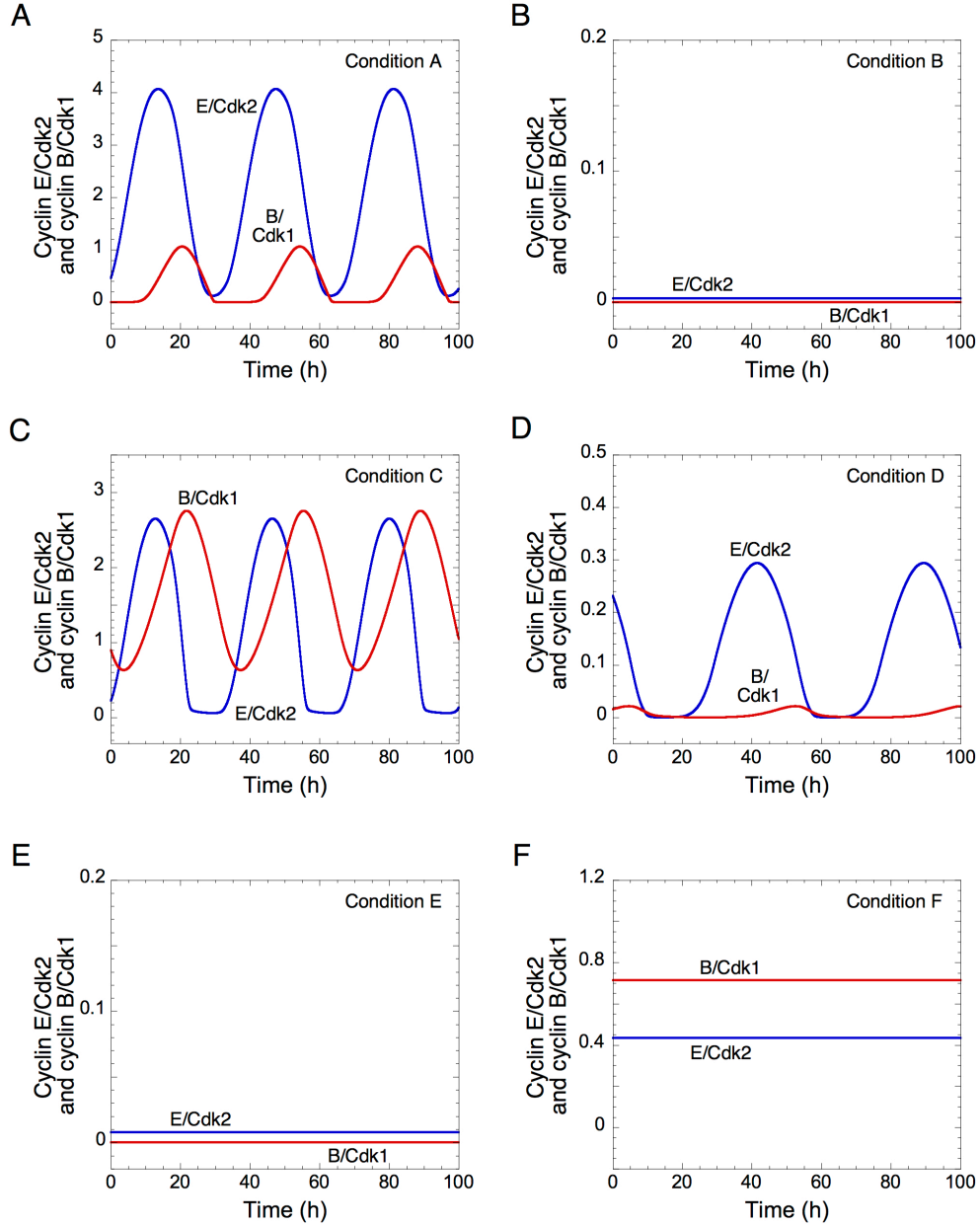


Figure S2. Cell proliferation vs cell cycle arrest mediated by Let-7 and GF levels. The temporal evolutions of cyclin E/Cdk2 (blue) and cyclin B/Cdk1 (red) in panels A to F correspond to conditions A to F of panel A from Fig. 2. In A, $GF = 3$ and $V_{SLET7} = 0.25$; in B, $GF = 3$ and $V_{SLET7} = 2$; in C, $GF = 25$ and $V_{SLET7} = 0.25$; in D, $GF = 25$ and $V_{SLET7} = 1.6$; in E, $GF = 25$ and $V_{SLET7} = 5$; and in F, $GF = 70$ and $V_{SLET7} = 1.5$. Other parameter values are as in Table S5.

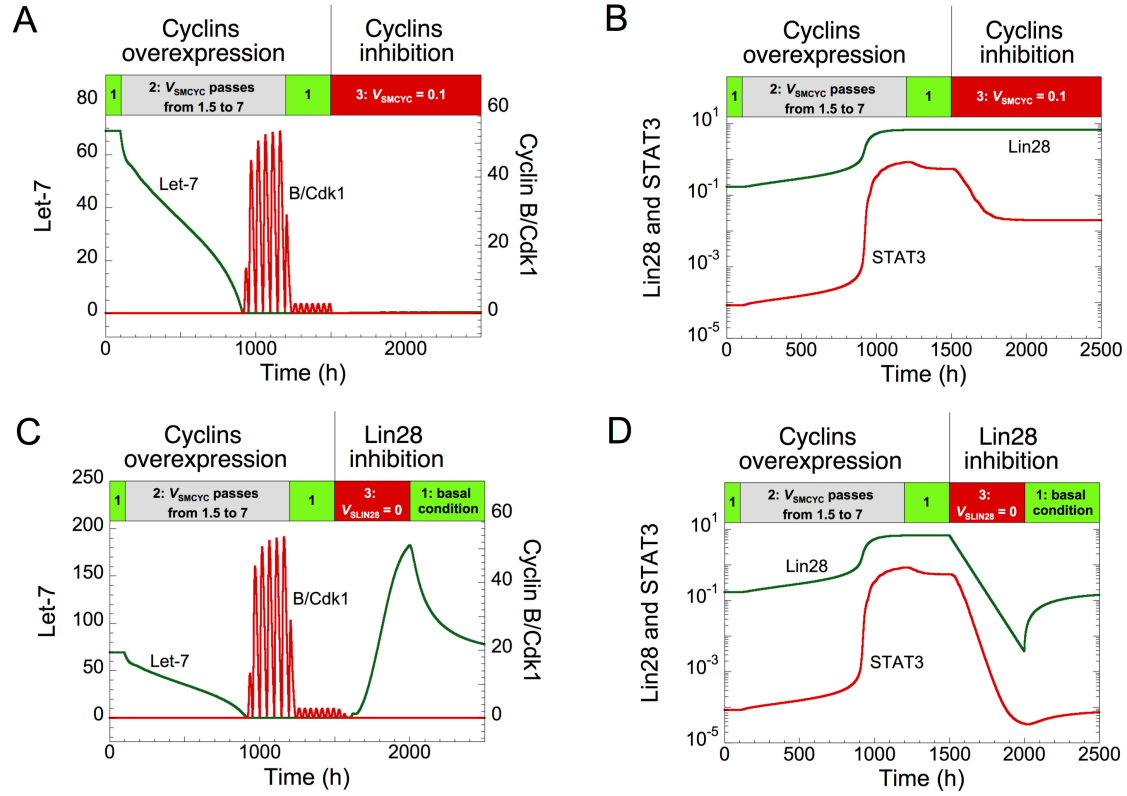


Figure S3. Effect of the Cdk network on the transformation network dynamics. (A, C) Temporal evolution of Let-7 and cyclin B/Cdk1. From a non-transformed and quiescent cell state, V_{SMCYC} changes from 1.5 to 7 (at $t = 100$ h), and promotes the epigenetic switch and cell proliferation. For $1200 \text{ h} < t < 1500 \text{ h}$, V_{SMCYC} is set back to its initial value ($= 1.5$), but cell proliferation and the transformed cell state are maintained, which is characterized by sustained oscillations in cyclin B/Cdk1 (with the same amplitude as in Fig. 1B) and low levels in Let-7. In A, at $t = 1500$ h, V_{SMCYC} changes from 1.5 to 0.1, preventing cell proliferation without recovering a non-transformed cell state since cyclin B/Cdk1 tends to a low stable steady state level, while Let-7 levels remain low. In C, for $1500 \text{ h} < t < 2000 \text{ h}$, the synthesis rate of Lin28, V_{SLIN28} is set to 0, allowing the recovery of a stable non-transformed state defined by high levels of Let-7 and impeding cell proliferation, characterized by a low, stable steady state, level of cyclin B/Cdk1. In C, for $t > 2000$ h, basal conditions are used. (B, D) Temporal evolution of Lin28 and STAT3 corresponds to conditions of panels A and C, respectively. Other parameter values are as in Table S5, which correspond to basal conditions.

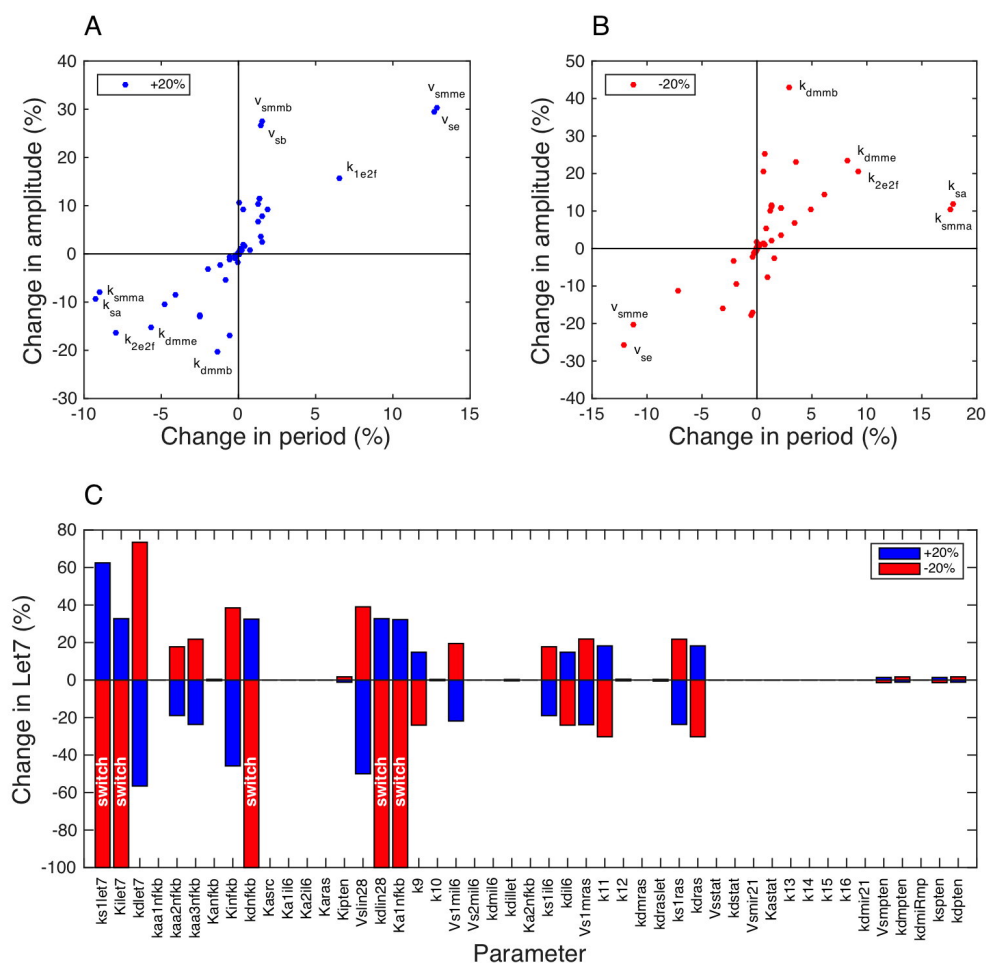


Figure S4. Sensitivity analysis. (A,B) Effect of changes in parameter values on the cell cycle. Each dot corresponds to an increase (A) or a decrease (B) of 20% of one parameter value. On the y-axis, 'amplitude' refers to the amplitude of Cyclin B/Cdk1 (variable Mb). The name of the parameters which influence the most the period and amplitude of the cell cycle are indicated. (C) Effect of changes in parameter values on the level of Let7 (in the condition where Let7 is activated and the cell cycle arrested). Blue/red bars refer respectively to an increase/decrease of 20% of one parameter value. Only parameters related to the transformation network are reported. Note that a decrease of some parameter values induces a switch of the transformation network (associated to the entry into a proliferative state). A change of 20% in the value of a single parameter of the cell cycle have virtually no effect of the level of Let7. As shown in Fig. S3, an over-expression of the cyclins may, however, activates the transformation network and trigger the cell cycle. Basal parameter values are as in Table S5.

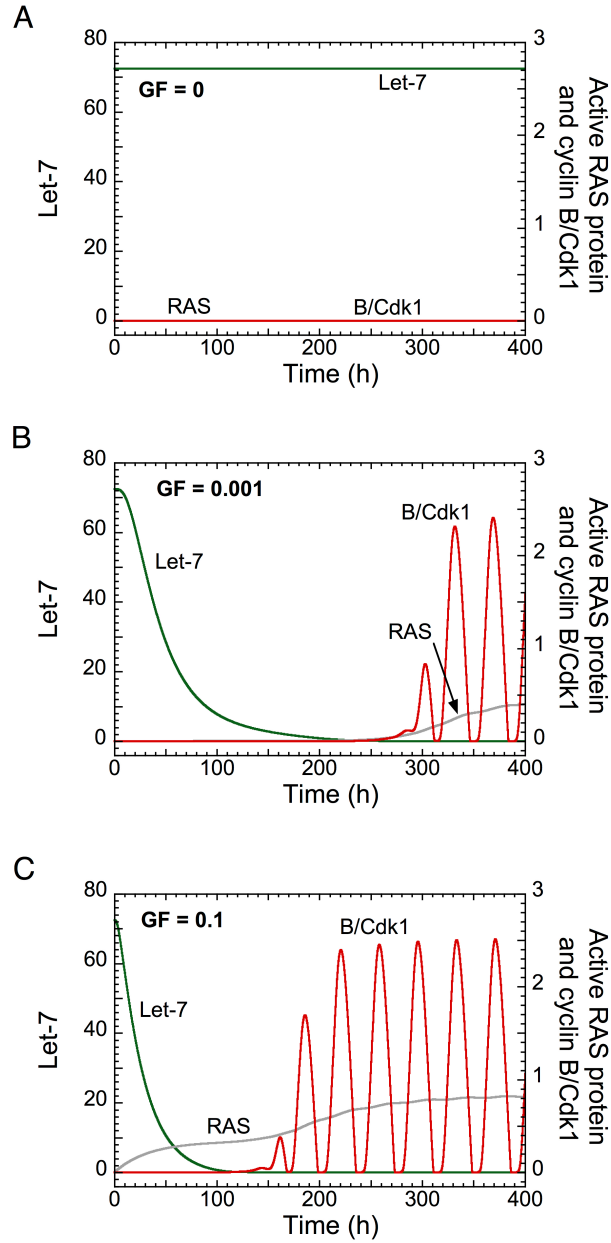


Figure S5. Growth factors couple transformation and proliferation networks in a Let-7-independent manner. Temporal evolution of Let-7 (green curve), the active form of RAS protein (grey curve) and cyclin B/Cdk1 (red curve) in the absence of GF (A), in the presence of low, $GF = 0.001$ in B, and higher levels of GF, $GF = 0.1$ in C. k_{S2RAS} in Eq. [24'] is equal to 0.1. Other parameter values and kinetic equations are as in Supplementary Information where Eq. [24] was replaced by Eq. [24'].

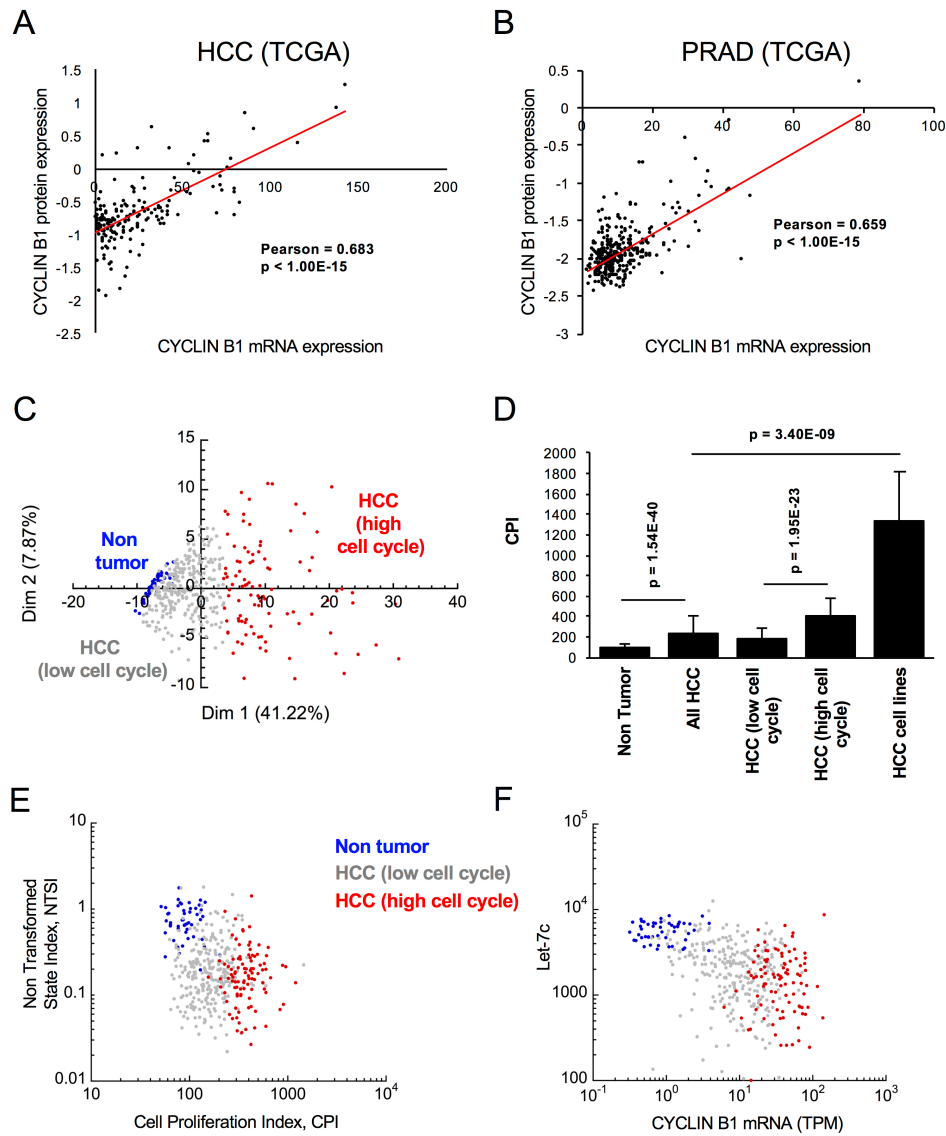


Figure S6. CPI correlates with cell cycle activation. (A, B) Expression levels of CYCLIN B1 protein (RPPA data, level 4, from the TCPA) as a function of CYCLIN B1 mRNA (TPM from TCGA) in the HCC cohort (A) and in prostate adenocarcinoma cohort (B). Each dot represents one sample in the cohort. For HCC cohort, $n = 177$, while for PRAD cohort, $n = 348$. (C) PCA analysis based on the mRNA expression of the cell cycle network components defined by KEGG. Blue dots : non tumor samples ($n = 50$), grey dots : HCC with low cell cycle activation ($n = 268$), red dots : 100 HCC samples with high cell cycle activation. (D) Mean value $\pm$ SD of CPI in non tumor ($n = 50$), in all HCC ($n = 368$), in HCC low ($n = 268$) or high cell cycle activation ($n = 100$), and in 20 HCC cell lines (<http://www.oasis-genomics.org/>). (E, F) Expression levels of NTSI *versus* CPI and Let-7c *versus* CYCLIN B1 mRNA in the HCC cohort.

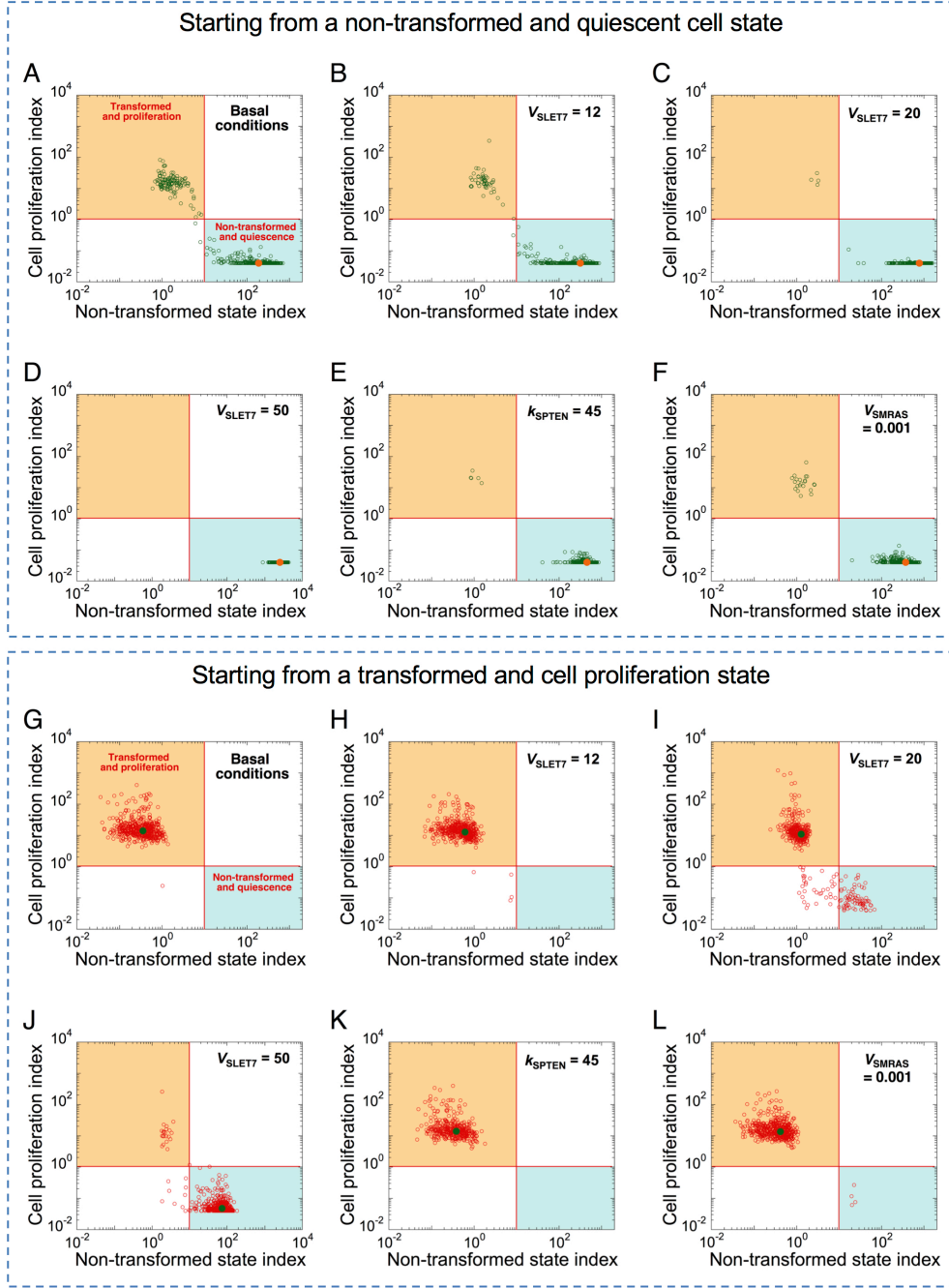


Figure S7. Oncogenes and tumor suppressors control the robustness of the networks dynamics. Starting from a non-transformed and quiescent cell state (A-F), or from a transformed and proliferating cell state (G-L), the cell proliferation index *versus* non-transformed state index is shown in a heterogeneous cell population with 25% of uniform random variation of all parameters of the model. In both cases, simulations are performed in the basal conditions (A and G); in the presence of various overexpression levels of Let-7: $V_{SLET7} = 12$ in B and H, 20 in C and I, and 50 in D and J; in the presence of PTEN overexpression, $k_{SPTEN} = 45$ in E and K, or in the presence of Ras inhibition, $V_{SMRAS} = 0.001$ instead of 0.006 in F and L. In each case, circles correspond to one cell in a population of 500 cells. The orange (A-F) and green dots (G-L) correspond to the levels of *CPI* and *NTSI* in the absence of random variation on parameters. Basal condition corresponds to the parameter values in Table S5.

Supplementary references

- Alexiades, M.R., and Cepko, C. (1996). Quantitative analysis of proliferation and cell cycle length during development of the rat retina. *Dev Dyn* 205, 293-307.
- Gerard, C., and Goldbeter, A. (2011). A skeleton model for the network of cyclin-dependent kinases driving the mammalian cell cycle. *Interface Focus* 1, 24-35.
- Gerard, C., Gonze, D., Lemaigre, F., and Novak, B. (2014). A model for the epigenetic switch linking inflammation to cell transformation: deterministic and stochastic approaches. *PLoS Comput Biol* 10, e1003455.
- Goldbeter, A., and Koshland, D.E., Jr. (1981). An amplified sensitivity arising from covalent modification in biological systems. *Proc Natl Acad Sci U S A* 78, 6840-6844.
- Iliopoulos, D., Hirsch, H.A., and Struhl, K. (2009). An epigenetic switch involving NF-kappaB, Lin28, Let-7 MicroRNA, and IL6 links inflammation to cell transformation. *Cell* 139, 693-706.
- Le, S.J., J.; Husson, F. (2008). FactoMineR: An R Package for Multivariate Analysis. *Journal of Statistical Software* 25, 1-18.
- Schwanhauss, B., Busse, D., Li, N., Dittmar, G., Schuchhardt, J., Wolf, J., Chen, W., and Selbach, M. (2011). Global quantification of mammalian gene expression control. *Nature* 473, 337-342.
- Tyson, J.J., Chen, K.C., and Novak, B. (2003). Sniffers, buzzers, toggles and blinkers: dynamics of regulatory and signaling pathways in the cell. *Curr Opin Cell Biol* 15, 221-231.
- Wagner, A. (2005). Circuit topology and the evolution of robustness in two-gene circadian oscillators. *Proc Natl Acad Sci U S A* 102, 11775-11780.
- Wu, L., Fan, J., and Belasco, J.G. (2006). MicroRNAs direct rapid deadenylation of mRNA. *Proc Natl Acad Sci U S A* 103, 4034-4039.
- Zhang, Z., Qin, Y.W., Brewer, G., and Jing, Q. (2012). MicroRNA degradation and turnover: regulating the regulators. *Wiley Interdiscip Rev RNA* 3, 593-600.