Année de publication : 2019

Larissa Mourao, Guillaume Jacquemin, Mathilde Huyghe, Wojciech J Nawrocki, Naoual Menssouri, Nicolas Servant, Silvia Fre (2019 Jan 31)

Lineage tracing of Notch1-expressing cells in intestinal tumours reveals a distinct population of cancer stem cells.

Scientific reports : 888 : DOI : 10.1038/s41598-018-37301-3

Résumé

Colon tumours are hierarchically organized and contain multipotent self-renewing cells, called Cancer Stem Cells (CSCs). We have previously shown that the Notch1 receptor is expressed in Intestinal Stem Cells (ISCs); given the critical role played by Notch signalling in promoting intestinal tumourigenesis, we explored Notch1 expression in tumours. Combining lineage tracing in two tumour models with transcriptomic analyses, we found that Notch1+ tumour cells are undifferentiated, proliferative and capable of indefinite self-renewal and of generating a heterogeneous clonal progeny. Molecularly, the transcriptional signature of Notch1+ tumour cells highly correlates with ISCs, suggestive of their origin from normal crypt cells. Surprisingly, Notch1+ expression labels a subset of CSCs that shows reduced levels of Lgr5, a reported CSCs marker. The existence of distinct stem cell populations within intestinal tumours highlights the necessity of better understanding their hierarchy and behaviour, to identify the correct cellular targets for therapy.

Année de publication : 2018

Veronica Rodilla, Silvia Fre (2018 Nov 1)

Cellular Plasticity of Mammary Epithelial Cells Underlies Heterogeneity of Breast Cancer.

Biomedicines : DOI : 10.3390/biomedicines6040103

Résumé

The hierarchical relationships between stem cells, lineage-committed progenitors, and differentiated cells remain unclear in several tissues, due to a high degree of cell plasticity, allowing cells to switch between different cell states. The mouse mammary gland, similarly to other tissues such as the prostate, the sweat gland, and the respiratory tract airways, consists of an epithelium exclusively maintained by unipotent progenitors throughout adulthood. Such unipotent progenitors, however, retain a remarkable cellular plasticity, as they can revert to multipotency during epithelial regeneration as well as upon oncogene activation. Here, we revise the current knowledge on mammary cell hierarchies in light of the most recent lineage tracing studies performed in the mammary gland and highlight how stem cell differentiation or reversion to multipotency are at the base of tumor development and progression. In addition, we will discuss the current knowledge about the interplay between tumor cells of origin and defined genetic mutations, leading to different tumor types, and its implications in choosing specific therapeutic protocols for breast cancer

patients.

Anna M Lilja, Veronica Rodilla, Mathilde Huyghe, Edouard Hannezo, Camille Landragin, Olivier Renaud, Olivier Leroy, Steffen Rulands, Benjamin D Simons, Silvia Fre (2018 May 23)
Clonal analysis of Notch1-expressing cells reveals the existence of unipotent stem cells that retain long-term plasticity in the embryonic mammary gland.
Nature cell biology : DOI : 10.1038/s41556-018-0108-1

Résumé

Recent lineage tracing studies have revealed that mammary gland homeostasis relies on unipotent stem cells. However, whether and when lineage restriction occurs during embryonic mammary development, and which signals orchestrate cell fate specification, remain unknown. Using a combination of in vivo clonal analysis with whole mount immunofluorescence and mathematical modelling of clonal dynamics, we found that embryonic multipotent mammary cells become lineage-restricted surprisingly early in development, with evidence for unipotency as early as E12.5 and no statistically discernable bipotency after E15.5. To gain insights into the mechanisms governing the switch from multipotency to unipotency, we used gain-of-function Notch1 mice and demonstrated that Notch activation cell autonomously dictates luminal cell fate specification to both embryonic and basally committed mammary cells. These functional studies have important implications for understanding the signals underlying cell plasticity and serve to clarify how reactivation of embryonic programs in adult cells can lead to cancer.

Read the back to back *News and Views*

Année de publication : 2015

Michel Wassef, Veronica Rodilla, Aurélie Teissandier, Bruno Zeitouni, Nadege Gruel, Benjamin Sadacca, Marie Irondelle, Margaux Charruel, Bertrand Ducos, Audrey Michaud, Matthieu Caron, Elisabetta Marangoni, Philippe Chavrier, Christophe Le Tourneau, Maud Kamal, Eric Pasmant, Michel Vidaud, Nicolas Servant, Fabien Reyal, Dider Meseure, Anne Vincent-Salomon, Silvia Fre, Raphaël Margueron (2015 Dec 6)
Impaired PRC2 activity promotes transcriptional instability and favors breast tumorigenesis.
Genes & development : 2547-62 : DOI : 10.1101/gad.269522.115

Résumé

Alterations of chromatin modifiers are frequent in cancer, but their functional consequences often remain unclear. Focusing on the Polycomb protein EZH2 that deposits the H3K27me3 (trimethylation of Lys27 of histone H3) mark, we showed that its high expression in solid tumors is a consequence, not a cause, of tumorigenesis. In mouse and human models, EZH2

is dispensable for prostate cancer development and restrains breast tumorigenesis. High EZH2 expression in tumors results from a tight coupling to proliferation to ensure H3K27me3 homeostasis. However, this process malfunctions in breast cancer. Low EZH2 expression relative to proliferation and mutations in Polycomb genes actually indicate poor prognosis and occur in metastases. We show that while altered EZH2 activity consistently modulates a subset of its target genes, it promotes a wider transcriptional instability. Importantly, transcriptional changes that are consequences of EZH2 loss are predominantly irreversible. Our study provides an unexpected understanding of EZH2's contribution to solid tumors with important therapeutic implications.

María Elena Fernández-Sánchez, Sandrine Barbier, Joanne Whitehead, Gaëlle Béalle, Aude Michel, Heldmuth Latorre-Ossa, Colette Rey, Laura Fouassier, Audrey Claperon, Laura Brullé, Elodie Girard, Nicolas Servant, Thomas Rio-Frio, Hélène Marie, Sylviane Lesieur, Chantal Housset, Jean-Luc Gennisson, Mickaël Tanter, Christine Ménager, Silvia Fre, Sylvie Robine, Emmanuel Farge (2015 Jul 2)

Mechanical induction of the tumorigenic β-catenin pathway by tumour growth pressure.

Nature : 92-5 : DOI : 10.1038/nature14329

Résumé

The tumour microenvironment may contribute to tumorigenesis owing to mechanical forces such as fibrotic stiffness or mechanical pressure caused by the expansion of hyper-proliferative cells. Here we explore the contribution of the mechanical pressure exerted by tumour growth onto non-tumorous adjacent epithelium. In the early stage of mouse colon tumour development in the Notch(+)Apc(+/1638N) mouse model, we observed mechanistic pressure stress in the non-tumorous epithelial cells caused by hyper-proliferative adjacent crypts overexpressing active Notch, which is associated with increased Ret and β-catenin signalling. We thus developed a method that allows the delivery of a defined mechanical pressure in vivo, by subcutaneously inserting a magnet close to the mouse colon. The implanted magnet generated a magnetic force on ultra-magnetic liposomes, stabilized in the mesenchymal cells of the connective tissue surrounding colonic crypts after intravenous injection. The magnetically induced pressure quantitatively mimicked the endogenous early tumour growth stress in the order of 1,200 Pa, without affecting tissue stiffness, as monitored by ultrasound strain imaging and shear wave elastography. The exertion of pressure mimicking that of tumour growth led to rapid Ret activation and downstream phosphorylation of β-catenin on Tyr654, imparing its interaction with the E-cadherin in adherens junctions, and which was followed by β-catenin nuclear translocation after 15 days. As a consequence, increased expression of β-catenin-target genes was observed at 1 month, together with crypt enlargement accompanying the formation of early tumorous aberrant crypt foci. Mechanical activation of the tumorigenic β-catenin pathway suggests unexplored modes of tumour propagation based on mechanical signalling pathways in healthy epithelial cells surrounding the tumour, which may contribute to tumour heterogeneity.

Veronica Rodilla, Alessandro Dasti, Mathilde Huyghe, Daniel Lafkas, Cécile Laurent, Fabien Reyal, Silvia Fre (2015 Feb 17)

Luminal progenitors restrict their lineage potential during mammary gland development.

PLoS biology : e1002069 : DOI : 10.1371/journal.pbio.1002069

Résumé

The hierarchical relationships between stem cells and progenitors that guide mammary gland morphogenesis are still poorly defined. While multipotent basal stem cells have been found within the myoepithelial compartment, the in vivo lineage potential of luminal progenitors is unclear. Here we used the expression of the Notch1 receptor, previously implicated in mammary gland development and tumorigenesis, to elucidate the hierarchical organization of mammary stem/progenitor cells by lineage tracing. We found that Notch1 expression identifies multipotent stem cells in the embryonic mammary bud, which progressively restrict their lineage potential during mammary ductal morphogenesis to exclusively generate an ERαneg luminal lineage postnatally. Importantly, our results show that Notch1-labelled cells represent the alveolar progenitors that expand during pregnancy and survive multiple successive involutions. This study reveals that postnatal luminal epithelial cells derive from distinct self-sustained lineages that may represent the cells of origin of different breast cancer subtypes.

Année de publication : 2014

Markus Germann, Huiling Xu, Jordane Malaterre, Shienny Sampurno, Mathilde Huyghe, Dane Cheasley, Silvia Fre, Robert G Ramsay (2014 Oct 8)

Tripartite interactions between Wnt signaling, Notch and Myb for stem/progenitor cell functions during intestinal tumorigenesis.

Stem cell research : 355-66 : DOI : 10.1016/j.scr.2014.08.002

Résumé

Deletion studies confirm Wnt, Notch and Myb transcriptional pathway engagement in intestinal tumorigenesis. Nevertheless, their contrasting and combined roles when activated have not been elucidated. This is important as these pathways are not ablated but rather are aberrantly activated during carcinogenesis. Using ApcMin/+ mice as a source of organoids we documented their transition, on a clone-by-clone basis, to cyst-like spheres with constitutively activated Wnt pathway, increased self-renewal and growth and reduced differentiation. We then looked at this transition when Myb and/or Notch1 are activated. Activated Notch promoted cyst-like organoids. Conversely growth and propagation of cyst-like, but not normal organoids were Notch-independent. Activated Myb promoted normal, but not cyst-like organoids. Interestingly the Wnt, Notch and Myb pathways were all involved in regulating the expression of the intestinal stem cell (ISC) gene Lgr5 in organoids, while ISC gene and Notch target Olfm4 was dominantly repressed by Wnt. These findings parallel mouse intestinal adenoma formation where Notch promoted the initiation, but not growth, of

Wnt-driven Olfm4-repressed colon tumors. Also Myb was essential for colon tumor initiation and collateral mouse pathologies. These data reveal the complex interplay and hierarchy of transcriptional networks that operate in ISCs and uncover a shift in pathway-dependencies during tumor initiation.

Maia Chanrion, Inna Kuperstein, Cédric Barrière, Fatima El Marjou, David Cohen, Danijela Vignjevic, Lev Stimmer, Perrine Paul-Gilloteaux, Ivan Bièche, Silvina Dos Reis Tavares, Giuseppe-Fulvio Boccia, Wulfran Cacheux, Didier Meseure, Silvia Fre, Loredana Martignetti, Patricia Legoix-Né, Elodie Girard, Luc Fetler, Emmanuel Barillot, Daniel Louvard, Andreï Zinovyev, Sylvie Robine (2014 Apr 9)

Concomitant Notch activation and p53 deletion trigger epithelial-to-mesenchymal transition and metastasis in mouse gut.

Nature communications : 5005 : DOI : 10.1038/ncomms6005

Résumé

Epithelial-to-mesenchymal transition-like (EMT-like) is a critical process allowing initiation of metastases during tumour progression. Here, to investigate its role in intestinal cancer, we combine computational network-based and experimental approaches to create a mouse model with high metastatic potential. Construction and analysis of this network map depicting molecular mechanisms of EMT regulation based on the literature suggests that Notch activation and p53 deletion have a synergistic effect in activating EMT-like processes. To confirm this prediction, we generate transgenic mice by conditionally activating the Notch1 receptor and deleting p53 in the digestive epithelium (NICD/p53(-/-)). These mice develop metastatic tumours with high penetrance. Using GFP lineage tracing, we identify single malignant cells with mesenchymal features in primary and metastatic tumours in vivo. The development of such a model that recapitulates the cellular features observed in invasive human colorectal tumours is appealing for innovative drug discovery.

Année de publication : 2013

Daniel Lafkas, Veronica Rodilla, Mathilde Huyghe, Larissa Mourao, Hippokratis Kiaris, Silvia Fre (2013 Oct 7)

Notch3 marks clonogenic mammary luminal progenitor cells in vivo.

The Journal of cell biology : 47-56 : DOI : 10.1083/jcb.201307046

Résumé

The identity of mammary stem and progenitor cells remains poorly understood, mainly as a result of the lack of robust markers. The Notch signaling pathway has been implicated in mammary gland development as well as in tumorigenesis in this tissue. Elevated expression of the Notch3 receptor has been correlated to the highly aggressive « triple negative » human breast cancer. However, the specific cells expressing this Notch paralogue in the

mammary gland remain unknown. Using a conditionally inducible Notch3-CreERT2(SAT) transgenic mouse, we genetically marked Notch3-expressing cells throughout mammary gland development and followed their lineage in vivo. We demonstrate that Notch3 is expressed in a highly clonogenic and transiently quiescent luminal progenitor population that gives rise to a ductal lineage. These cells are capable of surviving multiple successive pregnancies, suggesting a capacity to self-renew. Our results also uncover a role for the Notch3 receptor in restricting the proliferation and consequent clonal expansion of these cells.

Année de publication : 2011

Silvia Fre, Edouard Hannezo, Sanja Sale, Mathilde Huyghe, Daniel Lafkas, Holger Kissel, Angeliki Louvi, Jeffrey Greve, Daniel Louvard, Spyros Artavanis-Tsakonas (2011 Jul 5)

Notch lineages and activity in intestinal stem cells determined by a new set of knock-in mice.

PloS one : e25785 : DOI : 10.1371/journal.pone.0025785

Résumé

The conserved role of Notch signaling in controlling intestinal cell fate specification and homeostasis has been extensively studied. Nevertheless, the precise identity of the cells in which Notch signaling is active and the role of different Notch receptor paralogues in the intestine remain ambiguous, due to the lack of reliable tools to investigate Notch expression and function in vivo. We generated a new series of transgenic mice that allowed us, by lineage analysis, to formally prove that Notch1 and Notch2 are specifically expressed in crypt stem cells. In addition, a novel Notch reporter mouse, Hes1-EmGFP(SAT), demonstrated exclusive Notch activity in crypt stem cells and absorptive progenitors. This roster of knock-in and reporter mice represents a valuable resource to functionally explore the Notch pathway in vivo in virtually all tissues.

Silvia Fre, Allison Bardin, Sylvie Robine, Daniel Louvard (2011 May 13)

Notch signaling in intestinal homeostasis across species: the cases of Drosophila, Zebrafish and the mouse.

Experimental cell research : 2740-7 : DOI : 10.1016/j.yexcr.2011.06.012

Résumé

Notch signaling has been recently shown to have a fundamental role in stem cell maintenance and control of proper homeostasis in the intestine of different species. Here, we briefly review the current literature on Notch signals in the intestine of Drosophila, Zebrafish and the mouse, and try to highlight conserved and divergent Notch functions across species. Notch signals show a remarkably conserved role in skewing cell fate choices in intestinal lineages throughout evolution. Genetic analysis demonstrates that loss of Notch signaling invariably leads to increased numbers of secretory cells and loss of enterocytes, while gain

of Notch function will completely block secretory cell differentiation. Finally, we discuss the potential contribution of Notch signaling to the initiation of colorectal cancer by controlling the maintenance of the undifferentiated state of intestinal neoplastic cells and speculate on the therapeutic consequences of affecting cancer stem cells.

Année de publication : 2009

Silvia Fre, S K Pallavi, Mathilde Huyghe, Marick Laé, Klaus-Peter Janssen, Sylvie Robine, Spyros Artavanis-Tsakonas, Daniel Louvard (2009 Feb 27)

Notch and Wnt signals cooperatively control cell proliferation and tumorigenesis in the intestine.

Proceedings of the National Academy of Sciences of the United States of America : 6309-14 : DOI : 10.1073/pnas.0900427106

Résumé

Notch and Wnt signals play essential roles in intestinal development and homeostasis, yet how they integrate their action to affect intestinal morphogenesis is not understood. We examined the interplay between these two signaling pathways in vivo, by modulating Notch activity in mice carrying either a loss- or a gain-of-function mutation of Wnt signaling. We find that the dramatic proliferative effect that Notch signals have on early intestinal precursors requires normal Wnt signaling, whereas its influence on intestinal differentiation appears independent of Wnt. Analogous experiments in Drosophila demonstrate that the synergistic effects of Notch and Wnt are valid across species. We also demonstrate a striking synergy between Notch and Wnt signals that results in inducing the formation of intestinal adenomas, particularly in the colon, a region rarely affected in available mouse tumor models, but the primary target organ in human patients. These studies thus reveal a previously unknown oncogenic potential of Notch signaling in colorectal tumorigenesis that, significantly, is supported by the analysis of human tumors. Importantly, our experimental evidence raises the possibility that Notch activation might be an essential initial event triggering colorectal cancer.

Année de publication : 2008

Silvia Fre, Danijela Vignjevic, Marie Schoumacher, Shannon L Duffy, Klaus-Peter Janssen, Sylvie Robine, Daniel Louvard (2008 Jul 16)

Epithelial morphogenesis and intestinal cancer: new insights in signaling mechanisms.

Advances in cancer research : 85-111 : DOI : 10.1016/S0065-230X(08)00003-1

Résumé

In this review, the major signal transduction pathways that have been shown to play an important role in intestinal homeostasis are highlighted. Each of them, the Wnt, Notch,

Hedgehog, and Bone Morphogenetic Protein, as well as growth-factor regulated Receptor Tyrosine Kinases are depicted with a special emphasis through their involvement in stem cell maintenance and their role in intestinal tumorigenesis. Finally, we discuss recent data on the final steps of tumor progression, notably the formation of distant metastases. This multistep process is highly complex and still far from being understood while being of major importance for the survival of patients with digestive cancer.

Année de publication : 2007

D Vignjevic, S Fre, D Louvard, S Robine (2007 Jan 6)
Conditional mouse models of cancer.
Handbook of experimental pharmacology : 263-87

Résumé

The development of inducible and conditional technologies allowed us to generate transgenic mouse models that faithfully recapitulate human tumorigenesis. It is possible to control, in time and space, the development of tumors in almost every mouse tissue. The result is that now we have available mouse models for all major human cancers. Novel noninvasive approaches to tumor imaging will enable us to follow tumor development and metastasis in vivo, as well as the effects of candidate therapeutic drugs. Such new generation tumor models, which accurately emulate the disease state in situ, should provide a useful platform with which to experimentally test drugs targeted to specific gene products, or combinations of genes that control rate-limiting steps of tumor development. In this review, we focus on the different mouse models for colon cancer.

Année de publication : 2005

Silvia Fre, Mathilde Huyghe, Philippos Mourikis, Sylvie Robine, Daniel Louvard, Spyros Artavanis-Tsakonas (2005 Jun 16)
Notch signals control the fate of immature progenitor cells in the intestine.
Nature : 964-8

Résumé

The Notch signalling pathway plays a crucial role in specifying cellular fates in metazoan development by regulating communication between adjacent cells. Correlative studies suggested an involvement of Notch in intestinal development. Here, by modulating Notch activity in the mouse intestine, we directly implicate Notch signals in intestinal cell lineage specification. We also show that Notch activation is capable of amplifying the intestinal progenitor pool while inhibiting cell differentiation. We conclude that Notch activity is required for the maintenance of proliferating crypt cells in the intestinal epithelium.