Cell Cycle 9:7, 1231-1240; April 1, 2010; © 2010 Landes Bioscience

Cell Cycle News & Views

Cyclins A and E trigger DNA damage

Comment on: Tane S, et al. Cell Cycle 2009; 8:3900–3.

Junko Odajima, Ilona Kalaszczynska and Piotr Sicinski; Dana-Farber Cancer Institute; and Harvard Medical School; Boston, MA USA;
Email: Peter_sicinski@dfci.harvard.edu

Cyclins represent regulatory subunits of the cyclin-dependent kinases (Cdks), a group of serine/threonine kinases that are crucial cell cycle engines. Expression of cyclins at the right cell cycle phase is essential for normal cell cycle progression. Indeed, deregulated expression of cyclins can induce abnormal proliferation, and overexpression of particular cyclins frequently correlates with aggressiveness of human cancers and with poor prognosis.[1]

DNA replication is a central event of the cell cycle that is tightly regulated by cyclin-Cdk complexes. Cyclin E is a key molecular player in the assembly of pre-replication complexes (pre-RCs) at origins of DNA replication (also referred to as "origin licensing"). When cells enter the cell cycle from quiescence, the pre-RCs are assembled in the G_1 phase in a stepwise fashion. The six-subunit initiator proteins (Orc1-6) bind to origins of replication and serve as a molecular landing pad onto which Cdc6, Cdt1 and Mcm proteins are sequentially recruited.[2] During this pre-RC assembly, cyclin E-Cdk2 phosphorylates Cdc6 and stabilizes it, thereby preventing its degradation via the APC/C.[3] Moreover, cyclin E-Cdk2 contributes to Mcm loading by promoting the accumulation of Cdc7, which then phosphorylates Mcm2.[4] Cyclin E has also been shown to stimulate Mcm loading in a kinase-independent fashion.[5] Additionally, cyclin E-Cdk2 plays an important role in recruitment of Cdc4[5] and GINS complex to the origins and in stimulating DNA helicase activity of Mcm proteins.[6]

In contrast, cyclin A is thought to play a negative role as a licensing inhibitor, preventing DNA re-replication. Cyclin A-Cdk2 phosphorylates Cdt1, which in turn inhibits Cdt1's DNA binding activity and targets Cdt1 for proteolysis by SCF^{skp2}.[7] Cyclin A-Cdk2 also targets Cdc6 and Orc1 upon entry of cells into the S phase, thereby triggering the degradation of Cdc6 and Orc1.[7] In addition, cyclin A-Cdk2 can phosphorylate Mcm4, and this inhibits DNA helicase activity of Mcm complex.[8] These cyclin A-dependent events ensure that each of DNA replication origins fires only once per cell cycle.

Aberrant activation of cyclin E- or cyclin A-Cdk complexes has been reported to trigger genomic instability,[9,10] one of the hallmarks of cancer cells. Overexpression or premature expression of cyclins E or A in mammalian cells causes impaired loading of Mcm proteins onto chromatin.[8,11] This in turn impairs origin firing and leads to prolonged S phase progression or cell cycle arrest at the G_1/S boundary. It has been hypothesized that cells with reduced number of active replication origins are more prone to have stalled replication forks and to undergo double-strand DNA breaks (DSBs), which may eventually lead to genomic instability.[11]

©2010 Landes Bioscience. Do not distribute.

In a recent issue of *Cell Cycle*,[12] Tane and Chibazakura report that overexpression of cyclins A or E in mammalian cells indeed induces DSBs. Using both human and mouse fibroblast cell lines and primary MEFs, the authors showed that forced expression of cyclins A or E leads to extensive γ-H2AX focus formation, an indicator of DSBs. This DSB induction by cyclins A and E seems to be kinase-dependent, because the number of γ-H2AX foci is reduced in the presence of $p21^{Cip1/Waf1}$ or dominant-negative Cdk2. These intriguing observations raise several important questions: (1) would overexpression of cyclin A or E have a similar effect on other cell types, for instance on epithelial cells from which cancers arise? (2) How high were the levels of overexpressed cyclins in the studies of Tane and Chibazakura, and how do these levels compare to those seen in cancer cells with cyclin A/E overexpression? (3) Is rescue of DSBs by $p21^{Cip1/Waf1}$ or dominant-negative Cdk2 a direct effect due to inhibition of cyclin A/E-associated Cdk kinase activity or an indirect effect due to a possible cell cycle arrest caused by these Cdk inhibitors?

Perhaps the most pressing question is how exactly cyclins A and E trigger DNA damage. Stated differently, does the prolonged S phase progression in cells overexpressing cyclin A or E represent a cause or a consequence of the increased DSBs? Tane and Chibazakura reported that cyclin A-associated Cdks do not phosphorylate histone H2AX in vitro, indicating that histone H2AX is not a direct substrate of Cdks. Hence, it is likely that DSBs are caused via unscheduled phosphorylation of some other Cdk target protein(s). This is consistent with the work recently reported by Wheeler et al.,[8] suggesting that premature activation of cyclin A-Cdk2 phosphorylates Cdt1 and triggers its unscheduled degradation pathway. This Cdt1 deficiency, in turn, impairs Mcm recruitment to replication origins resulting in a failure to assemble active pre-RC. As reduced number of active replication origins can potentially lead to DSBs,[13] further studies are required to determine whether DSBs and insufficient number of active replication origins coexist in cells overexpressing cyclin E or A.

Of note, Brca2, a protein involved in DNA repair was also shown to be phosphorylated at the carboxy-terminus by Cdks, and this phosphorylation inhibited interaction of Brca2 with Rad51.[14] Further studies are also needed to determine whether the effects observed by Tane and Chibazakura are related to Brca2 phosphorylation.

Whatever is the exact molecular mechanism, the study of Tane and Chibazakura demonstrates a novel function of overexpressed cyclins E and A in triggering DNA damage. The checkpoint system that leads to cell cycle arrest, senescence or apoptosis is likely activated by DSBs in cells overexpressing cyclin E or A, which sounds at odds with the known pro-oncogenic function of these proteins. It is likely that cancer cells develop ways to overcome this effect of cyclins possibly by disabling DNA damage checkpoint proteins. In this respect, it will be interesting to compare the effects of cyclin E/A overexpression in p53 wild-type versus mutant cells.

References

1. Malumbres M, et al. Nat Rev Cancer 2001; 1:222-31.
2. Prasanth SG, et al. Philos Trans R Soc Lond B Biol Sci

2004; 359:7-16.
3. Mailand N, et al. Cell 2005;122:915-26.
4. Chuang LC, et al. Mol Cell 2009; 206-16.
5. Geng Y, et al. Cell 2003;114:431-43.
6. Cook JG. Front Biosci 2009;14:5013-30.
7. Diffley JF. Curr Biol 2004; 14:R778-86.
8. Wheeler LW, et al. Cell Cycle 2008; 7:2179-88.
9. Spruck CH, et al. Nature 1999; 401:297-300.
10. Chibazakura T, et al. Proc Natl Acad Sci USA 2004;101:4465-70.
11. Ekholm-Reed S, et al. J Cell Biol 2004; 165:789-800.
12. Tane S, et al. Cell Cycle 2009; 8:3900-3.
13. Blow JJ, et al. Nat Rev Cancer 2008; 8:799-806.
14. Esashi F, et al. Nature 2005; 434, 598-604.

TACCing on new functions for the TSC2 tumor suppressor

Comment on: Gómez-Baldó L, et al. Cell Cycle 2010; 9:1143–55.

Erica A. Golemis; Fox Chase Cancer Center; Philadelphia, PA USA; Email: EA_Golemis@fccc.edu

Tuberous sclerosis complex (TSC) is a rare disease marked by a confusing range of clinical symptoms that can include hamartomas, neurological symptoms, renal cysts and (in females only) the pulmonary syndrome lymphangioleiomyomatosis (LAM). TSC arises from mutations in one of two genes: *TSC1*, encoding hamartin, and *TSC2*, encoding tuberin.[1-3] Although the mechanistic basis by which mutations in *TSC1* and *TSC2* induce disease was for a long time obscure, studies appearing over the past several years have identified multiple roles for their encoded proteins in restricting cell proliferation (reviewed in ref. 4). Among these, the role of a hamartin-tuberin heterodimer in restraining the action of the mammalian Target Of Rapamycin (mTOR) cell growth-promoting kinase has been the target of intense interest, and has led to promising therapeutic advances arising from the application of mTOR inhibitors to treat TSC in the clinic.[5] More recent studies have also shown the TSC proteins stabilize the cell cycle kinase inhibitor p27(Kip1) to limit cell cycle progression from G_1 to S (see ref. 6), suggesting an independent means by which mutation of *TSC* genes induces abnormal proliferation. However, some aspects of the pathological presentation of TSC have remained obscure. For example, mutations in *TSC2* are typically associated with more severe clinical symptoms than mutations in *TSC1*,[7] suggesting potentially broader action of tuberin than can be accounted for based solely on its role as a component of the tuberin-hamartin heterodimer.

In the last issue of *Cell Cycle*, work by Gómez-Baldó et al. potentially identifies one such *TSC2*/tuberin-specific function.[8] The goal of this study was initially to illuminate the function of TACC3, a member of the Transforming Acidic Coiled-Coil domain family of proteins.[9] TACC proteins are evolutionarily conserved from yeast to humans. As their family name implies, changes in their expression have been associated with cell transformation, with each of the three human members of the family (TACC1-3) localized to genomic regions amplified in some cancers, and with changes in TACC expression noted even in tumors lacking obvious DNA rearrangements. The TACC proteins are apparently non-catalytic, but contain multiple protein interaction domains. Previously defined partners for the interaction domains include notably ch-TOG/CKAP1 and Aurora kinases. These evolutionarily conserved interactors connect TACC proteins to regulation of processes including centrosome-dependent microtubule assembly and chromosomal alignment in mitosis, and appropriate completion of cytokinesis.[9,10] The present work began by utilizing extensive two-hybrid screening and network construction to more fully analyze the scope of TACC3 functional associations. While a number of previously known partners were isolated, as well as new partners plausibly linked to TACC3 functions at centrosomes and microtubules, one of the most intriguing hits was *TSC2*/tuberin.[8]

In their study, Gómez-Baldó et al. demonstrate that intracellular pools of TACC3 and tuberin (but not hamartin) localize to the nuclear membrane and copurify with the nuclear membrane component lamin-A, show that tuberin binds directly to the nuclear pore component NUP62, and also find that siRNA depletion of tuberin or TACC3 induces striking defects in nuclear morphology. In further work, they establish that TACC3 helps anchor a mitotically-phosphorylated pool of tuberin to the spindle poles in mitosis, and to the intercellular bridge in cytokinesis. They again use siRNA to demonstrate that depletion of these proteins leads to abnormalities of post-mitotic abscission and increased numbers of binucleate cells, and that depletion of TACC3 or tuberin leads to the triggering of a CHFR-dependent mitotic checkpoint. Although lesions in TSC1/hamartin have also been linked to changes in centrosomes and mitotic-specific regulation, to date, the mechanisms involved appear to be distinct, involving instead associations with Polo-like kinases (PLKs).[11,12]

Finally, the authors identify a number of new TACC3 interactors that point to new functions for this protein that may be relevant to TSC2 biology. As one example, they have now identified interactions between TACC3 and CP110 and CEP164, centrosomal proteins that also regulate formation of the primary cell cilium. The cilium provides a docking platform for receptors for Hedgehog and other cell-extrinsic growth-regulatory signals. Interestingly, both TSC2[13,14] and the TACC3 partner Aurora-A[15] regulate ciliary dynamics, while Aurora-A is also a direct target of CHFR in checkpoint initiation[16] and has broad action in regulation of centrosomally-anchored signaling functions.[17] These connections raise the possibility that TSC2/tuberin is part of a signaling network that controls cytoskeletal dynamics and ciliary-based signaling systems in addition to better-studied roles with TSC1/hamartin. Such long reach would certainly help explain the more severe presentation of TSC cases derived from TSC2 mutations. Overall, the study beautifully exemplifies the value of considering extended interaction networks in considering complex protein functions in disease, and joins with other such studies (see ref. 18) in identifying links between centrosomal defects and cancer.

©2010 Landes Bioscience. Do not distribute.

References

1. Consortium. Cell 1993; 75:1305-15.
2. van Slegtenhorst M, et al. Science 1997; 277:805-8.
3. Smolarek TA, et al. Am J Hum Genet 1998; 62:810-5.

4. Krymskaya VP, et al. Cell Cycle 2009; 8:403-13.
5. Sampson JR. Biochem Soc Trans 2009; 37:259-64.
6. Bhatia B, et al. Cell Cycle 2010; 9:1237-8.
7. Dabora SL, et al. Am J Hum Genet 2001; 68:64-80.
8. Gómez-Baldó L, et al. Cell Cycle 2010; 9:1143-55.
9. Peset I, et al. Trends Cell Biol 2008; 18:379-88.
10. Jeng JC, et al. Cell Cycle 2009; 8:3529-36.
11. Astrinidis A, et al. Hum Mol Genet 2006; 15:287-97.
12. Matthew EM, et al. Cell Cycle 2009; 8:4168-75.
13. Bonnet CS, et al. Hum Mol Genet 2009; 18:2166-76.
14. Hartman TR, et al. Hum Mol Genet 2009; 18:151-63.
15. Pugacheva EN, et al. Cell 2007; 129:1351-63.
16. Yu X, et al. Nat Genet 2005; 37:401-6.
17. Pugacheva EN, et al. Cell Cycle 2006; 5:384-91.
18. Pujana MA, et al. Nat Genet 2007; 39:1338-49.

Interaction of TACC3 and TSC2 at the nuclear envelope and mitotic structures

Comment on: Gómez-Baldó L, et al. Cell Cycle 2010; 9:1143–55.

Norman Ertych and Holger Bastians; Institute for Molecular Biology and Tumor Research; Philipps University Marburg; Marburg, Germany;
Email: bastians@imt.uni-marburg.de

The transforming acidic coiled-coil (TACC) proteins represent a protein family implicated in cancer with deregulated expression in various human tumors. While in fission yeast, nematodes, Drosophila and Xenopus only one familiy member is present (Alp7, TAC-1, D-TACC and Maskin, respectively) mammals possess three TACC proteins designated as TACC1, 2 and 3. During cell division, the TACC proteins exhibit characteristic localizations at centrosomes and spindle microtubules suggesting important functions during mitosis.[1] In fact, depletion of Maskin in Xenopus or loss of TACC3 in HeLa cells lead to destabilized microtubules and a reduced number of microtubules resulting in severe spindle assembly defects and chromosome misalignment.[2,3] This role of TACC3/maskin might be in particular mediated by its interaction with the microtubule stabilizing factor ch-TOG/XMAP215, which is recruited to centrosomes in a TACC dependent manner.[1,4]

While solid experimental support for the mitotic role of TACC proteins exist, little is known about the function of these proteins in interphase. Interestingly, in addition to its role in mitosis maskin has been characterized as a protein regulating mRNA translation and maturation in Xenopus oocytes. Maskin directly interacts with the translation initiation factor eIF4E, which contributes to an inhibition of translation, in particular of mRNAs with little or absent 3´-polyadenylation.[5,6] So far, a role in mRNA translation has not been established for TACC3 in mammals and it is not yet clear if and how regulation of translation relates to the mitotic roles established for TACC proteins.

In the most recent issue of *Cell Cycle*, Gómez-Baldó et al., performed large-scale yeast-two-hybrid screens to identify novel interaction partners for TACC3 and identified the tumor suppressor gene *TSC2*. *TSC2*, together with *TSC1* has been implicated in the tuberous sclerosis syndrome (TSC) and lymphangioleiomyomatosis (LAM), rare genetic diseases that cause benign tumors to grow in multiple tissues and to result in disorganized proliferation of smooth muscles in the lung, respectively. Hamartin and tuberin (encoded by *TSC1* and *TSC2*, respectively) form heterodimers and inhibit the mammlian target of rapamycin (mTOR).[7] Thus, *TSC1/TSC2* are implicated in regulation of mRNA translation by negatively regulating mTOR, the phosphorylation of eIF4E-BP1 and thus, the activation of the translation initiation factor eIF4E. Upon mitogenic stimuli, AKT can phosphorylate tuberin/TSC2 on serine-939, which relieves the inhibition of eIF4E.[8] Intriguingly, Gómez-Baldó and colleagues found that TACC3 and tuberin/TSC2 co-localize in interphase at the nuclear envelope and interact with the nuclear pore suggesting a possible novel role for TACC3-tuberin/TSC2 in the maintenance of nuclear envelope or in nucleo-cytoplasmic transport. This idea is further supported by the finding that loss of both *TSC2* or *TACC3* cause abnormalities in the structure of the nuclear envelope. The authors further found that the interaction of TACC3 and tuberin/TSC2 is even enhanced at the G_2/M transition and tuberin/TSC2 and TACC3 co-localize at centrosomes and spindles during the early phases of mitosis. However, during cytokinesis total tuberin/TSC2 and its serine-939 phosphorylated sub-pool localizes to the cleavage furrow with the latter being dependent on TACC3. This suggests a possible role for TACC3 in recruiting a sub-pool of tuberin/TSC2 to mitotic structures. Alternatively, depletion of TACC3 may lead to spindle destabilization,[2] which in turn might influence the localization of tuberin/TSC2 indirectly. The authors also suggest that both, TACC3 and tuberin/TSC2 might be required for timely execution of cytokinesis, but these results are in contrast to the observation that loss of TSC2 is associated with a significant higher proliferation rate.

Taken together, the work by Gómez-Baldó et al. uncovered a novel interaction of TACC3 with the tumor suppressor tuberin/TSC2, which might reflect new roles in the organization of the nuclear envelope and during cytokinesis. However, a more rigorous investigation of these putative functions will be required to further elucidate the roles of the TACC3-tuberin/TSC2 complex, both, in interphase and in mitosis. Since tuberin/TSC2 and TACC3/maskin have been implicated in mRNA translation by regulating the initiation factor eIF4E it is worthwhile to take an alteration in gene expression into account to explain the phenotypes observed in the absence of *TACC3* or *TSC2*. In this regard, it is interesting to note that the authors demonstrate that loss of TACC3 is associated with a loss of the mTOR mediated phosphorylation of the ribosomal S6 kinase and elevated protein levels of cyclin B1, which represents an established target for CPEB-maskin regulation in Xenopus egg extracts,[9] supporting a possible link between TACC3 and mRNA translation in mammalian cells.

©2010 Landes Bioscience. Do not distribute.

References

1. Peset I, et al. Trends Cell Biol 2008; 18:379-88.
2. Schneider L, et al. J Biol Chem 2007; 282:29273-83.
3. Kinoshita K, et al. J Cell Biol 2005; 170:1047-55.
4. Lee MJ, et al. Nat Cell Biol 2001; 3:643-9.
5. Groisman I, et al. Cold Spring Harb Symp Quant Biol 2001; 66:345-51.
6. Stebbins-Boaz B, et al. Mol Cell 1999; 4:1017-27.
7. Jozwiak J. Int J Cancer 2006; 118:1-5.
8. Inoki K, et al. Nat Cell Biol 2002; 4:648-57.
9. Groisman I, et al. Cell 2000; 103:435-47.

Tumor suppressor effect of the microRNA miR-519 is mediated via the mRNA-binding protein HuR

Comment on: Abdelmohsen K, et al. Cell Cycle 2010; 9:1354–9.

Ari Ristimäki; University of Helsinki; Helsinki, Finland; and, University of Oulu and Oulu University Hospital; Oulu, Finland; Email: ari.ristimaki@helsinki.fi

Deregulation of gene expression is a hallmark of the carcinogenic process. Gene expression is regulated by a series of events that can take place both at transcriptional and posttranscriptional levels. HuR (or HuA or ELAV1) is an mRNA-binding protein, and it is essential for placental and embryonic development[1] and for progenitor cell survival in an adult organism.[2] HuR is primarily localized in the nucleus, but it can shuttle between the nucleus and the cytoplasm.[3] In the nucleus, HuR binds to its target mRNAs, which are then transported to the cytoplasm, where HuR can stabilize the transcript and/or regulate its translation. Lopez de Silanes et al.[4] showed that cytoplasmic HuR expression is elevated in a variety of human carcinomas when compared to normal tissue specimens. Since the cytoplasmic HuR expression has been shown to be a prognostic marker in several cancer types,[5-7] these data strongly suggest that HuR plays an important role in carcinogenesis. This hypothesis is supported by experimental data, which show that HuR can modulate expression of several key factors related to tumor cell survival, growth and metastasis.[8]

HuR protein levels have recently been shown to be regulated by microRNAs through inhibition of its translation, which lead to suppression of cancer cell growth in vitro.[9,10] In this issue of *Cell Cycle*, Abdelmohsen et al. show that HuR protein is abundantly expressed in ovarian, lung and kidney carcinomas, while levels of miR-519 were reduced when compared to adjacent nonneoplastic tissues. This reverse expression pattern could indicate that there exists a direct link between the two factors in human cancers. Moreover, tumor growth could be affected by miR-519 via modulation of HuR expression. Indeed, it was shown in HeLa cells that miR-519 overexpression reduced tumor growth while suppression of the expression lead to larger tumors in athymic mice. To demonstrate link between the effect of miR-519 and HuR in this in vivo tumor model, HuR-silenced cells were shown to be unresponsive to miR-519. Future studies are needed to determine the mechanism of miR-519 suppression in tumor cells, and whether this marker can aid diagnosis and determination of prognosis of cancer patients.

©2010 Landes Bioscience. Do not distribute.

References

1. Katsanou V, et al. Mol Cell Biol 2009; 29: 2762-76.
2. Ghosh M, et al. J Clin Invest 2009; 119: 3530-43.
3. Brennan CM, et al. Cell Mol Life Sci 2001; 58: 266-77.
4. López de Silanes I, et al. Oncogene 2003; 22: 7146-54.
5. Erkinheimo T-L, et al. Cancer Res 2003; 63: 7591-4.
6. Heinonen M, et al. Cancer Res 2005; 65: 2157-61.
7. Mrena J, et al. Clin Cancer Res 2005; 11: 7362-8.
8. López de Silanes I, et al. RNA Biol 2005; 2: 11-3.
9. Abdelmohsen K, et al. Proc Natl Acad Sci USA 2008; 105: 20297-302.
10. Guo X, et al. RNA Biol 2009; 6: 575-83.

MicroRNA-RNA binding protein face-off in cancer

Comment on: Abdelmohsen K, et al. Cell Cycle 2010; 9:1354–9.

Xun Guo and Rebecca S. Hartley; University of New Mexico; Albuquerque, NM USA; Email: rhartley@salud.unm.edu

Post-transcriptional mechanisms regulate the expression of a wide variety of genes involved in cancer development, progression, and metastasis. RNA binding proteins (RBPs) are mediators of post-transcriptional control that are often disrupted in cancer.[1,2] RBPs bind regulatory elements most often located in the 3′ untranslated region (UTR) of target mRNAs to either increase or decrease their stability and translation. One RBP closely associated with tumorigenesis is mRNA stabilizing HuR. HuR expression level correlates with advancing stage of malignancy in cancers of the breast, colon, lung and ovary.[3] HuR regulates subsets of proteins that enhance proliferation, inhibit apoptosis, increase angiogenesis, reduce immune recognition and facilitate invasion and metastasis; the five major traits of cancer cells.[3] Despite its link to aggressive cancer, little is known about the mechanisms that upregulate HuR in cancer.

MicroRNAs are a class of small, non-coding RNAs that also post-transcriptionally control gene expression, almost always promoting decay and/or suppressing translation of their target mRNAs.[4] MicroRNAs play critical roles in cancer, functioning as oncogenes or tumor suppressors,[5] but there is limited understanding of their roles or targets in cancer. Two recent studies identified HuR as a target of two different microRNAs in human cancer cell lines.[6,7] HuR was translationally repressed by miR-519 and miR-125a. miR-125a is decreased in primary breast tumors[8] and in breast cancer cell lines that overexpress HuR,[6] but the expression level of either microRNA in HuR overexpressing tumors is not known. In a hallmark study in this issue of *Cell Cycle*, Abdelmohsen et al. show that miR-519 is decreased in tumors in which HuR is elevated and that miR-519 is a tumor suppressor that exerts its effects by repressing HuR.

The Gorospe group had previously shown that miR-519 repressed HuR translation, and by inhibiting HuR, miR-519 suppressed cell proliferation.[7] In the current study, they first analyzed the levels of miR-519 and HuR in paired human tumors and adjacent healthy tissues. When analyzed by western blots, HuR abundance was strikingly higher in all tumors examined (four ovarian, four kidney and three lung) as compared to the adjacent non-tumor sample (Figures 1-3). Consistent with prior studies,[7,9] HuR mRNA level was similar between the tumor and normal samples. This suggested that neither transcription nor mRNA stability contributed to HuR upregulation, and prompted analysis of miR-519 abundance. All miR-519 variants present in the individual tumors were decreased as compared to

normal tissues (Figures 1-3), suggesting that miR-519 could repress tumor growth by lowering HuR expression.

To test this, levels of miR-519 were modulated in human cervical cancer HeLa cells and their ability to form tumors in athymic mice tested. Tumor size was modulated by the expression level of miR-519, with cells overexpressing miR-519 giving rise to the smallest tumors and those with reduced miR-519 producing the largest tumors (Figure 4). To determine if HuR upregulation was involved in increased tumor growth, HuR was silenced in cells with reduced miR-519. Tumors in the HuR/miR-519 reduced group were significantly smaller than those in the miR-519 reduced group, indicating that the tumor-promoting effects of lowered miR-519 were dependent on HuR abundance.

These studies identify reduction of miR-519 as a key mechanism of HuR elevation in cancer and identify a key target for a tumor suppressor microRNA. Through its modulation of this one target, tumor apoptosis, proliferation, angiogenesis and invasion can be regulated (**Fig. 1**). This study highlights that combining RBP and microRNA expression patterns could have diagnostic, prognostic, and therapeutic potential in cancer. Yet, as both RBPs and microRNAs have many targets involved in these major cancer traits, a thorough knowledge of the targets and the interplay between RBPs and miRs is needed before intervening in aberrant post-transcriptional control. For example. HuR itself regulates microRNA binding. HuR inhibits c-Myc expression by recruiting let-7-loaded RISC to the c-Myc 3'UTR10.

Given that at least two different microRNA families target HuR for translational repression, redundant pathways appear to check HuR expression. Neither miR-519, whose target sequence is in the HuR coding region, nor miR-125a are consistently identified as targeting HuR by computational screening methods (such as TargetScan, miRanda and miRbase). This emphasizes that although in silico approaches provide important tools for microRNA target detection, key regulatory microRNAs can be overlooked by relying solely on these methods.

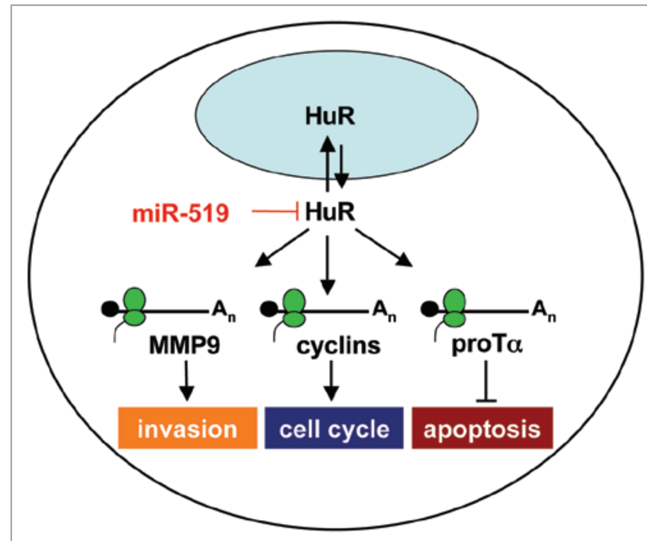

Figure 1. Model showing how miR-519 suppression of HuR can modulate cancer cell invasion, proliferation and apoptosis. HuR, a predominantly nuclear protein, shuttles to the cytoplasm to stabilize its mRNA targets, thus increasing their translation. Blue oval, cell nucleus; green circles, ribosomes; black circle, 7-methyl-guanosine cap; An, poly(A) tail; MMP9, matrix metalloprotease 9; proTα, prothymosin alpha.

©2010 Landes Bioscience. Do not distribute

Acknowledgments

Supported by the National Cancer Institute-National Institutes of Health (R01CA095898) and the UNM Cancer Center Inflammatory Breast Cancer Research Program. We thank Dr. David Port for his insightful comments (UCHSC, Denver, CO).

References

1. Lopez de Silanes I, et al. Cell Oncol 2007; 29:1-17.
2. Kim MY, et al. BMB Rep 2009; 42:125-30.
3. Abdelmohsen K, et al. WIREs RNA 2010; in press.
4. Chekulaeva M, et al. Curr Opin Cell Biol 2009; 21:452-60.
5. Garzon R, et al. Annu Rev Med 2009; 60:167-79.
6. Guo X, et al. RNA Biol 2009; 6:575-83.
7. Abdelmohsen K, et al. Proc Natl Acad Sci USA 2008; 105:20297-302.
8. Iorio MV, et al. Cancer Res 2005; 65:7065-70.
9. Brennan SE, et al. Cancer Res 2009; 69:5168-76.
10. Kim HH, et al. Genes Dev 2009; 23:1743-8.

A histone methylation code for SV40 minichromosomes

Comment on: Balakrishnan L, et al. Cell Cycle 2010; 9:1320–32.

Shumin Wu and Judd C. Rice; University of Southern California; Los Angeles, CA USA; Email: juddrice@usc.edu

Chromatin structure and function is dynamically regulated, in part, by the various post-translational modifications on the DNA-associated histone proteins. These histone modifications can occur alone or in combination at specific loci to create a "histone code" that is directly associated with a distinct DNA-templated process such as transcription or replication.[1] Although many studies throughout the last few decades have greatly increased our understanding of histone modification functions in eukaryotes, very little is known about the role of histone modifications within viral genomes. To address this, Milavetz and colleagues have been investigating alterations of histone modifications on the SV40 minichromosome during lytic infection of mammalian cells.[2] The circular double-stranded SV40 DNA is chromatinized in both infected cells and virions offering a relatively simple but powerful tool for examining histone modifications during viral infection. In this issue of *Cell Cycle*, Balakrishnan et al. continue to employ the SV40 minichromosome

to investigate a specific histone modification, H4 lysine 20 (H4K20) methylation, during lytic infection.

Lysine residues can accept up to three methyl groups and, therefore, can be mono- (me1), di- (me2) or trimethylated (me3).[3] Using antibodies that selectively discriminate between the different H4K20 methylated forms in chromatin immunoprecipitations (ChIPs), the authors determined that H4K20me1 was present on SV40 minichromosomes at all times tested post-infection. Although H4K20me1 coincided with RNA polymerase II during late infection suggesting a role in transcriptional activation, the authors found H4K20me1 broadly distributed throughout the SV40 minichromosome. H4K20me1 was also detected during encapsidation and in the newly assembled virions suggesting that this modification may be important for proper packaging of SV40. This is consistent with recent reports indicating that H4K20me1 is associated with chromatin condensation.[4]

It was previously postulated that H4K20me1 serves as the preferred substrate for higher degrees of H4K20 methylation.[5] Consistent with this theory, the authors provide evidence that H4K20me1 appears to be absent on H4K20me3-associated nucleosomes even though the levels of both modifications are highest during early infection. Interestingly, H4K20me1 remains on SV40 minichromosomes at later time points whereas H4K20me3 rapidly disappears strongly suggesting that these two histone modifications are differentially regulated and have distinct functions. What was the reason for the disappearance of H4K20me3? The authors noticed that the loss of H4K20me3 occurred within the same timeframe as the overall reduction of SV40 DNA suggesting a link between these two events. Indeed, the authors demonstrated that SV40 minichromosomes targeted for degradation were selectively enriched for H4K20me3 suggesting that H4K20me3 may serve as a rapid host defense mechanism, not unlike DNA methylation, to signal for inactivation and/or destruction of the viral DNA. This seems likely as mammalian H4K20me3 is enriched within repetitive and foreign DNA sequences typically associated with transcriptionally inert chromatin.[6]

One unexpected observation made by the authors was the near absence of H4K20me2 on the SV40 minichromosomes. This was surprising given that the majority of human H4 molecules are dimethylated and these are widely distributed throughout the genome.[7] The reasons for these differences remain unclear. Another interesting observation was the presence of H4K20me1 in replicating and newly replicated SV40 minichromosomes. This was unexpected as H4K20me1 levels in mammalian chromatin are lowest during DNA replication and maximal during mitosis.[8] The authors provide two possible explanations for this observation: that H4K20me1 is introduced into parental SV40 minichromosomes to permit the replication machinery to proceed through chromatin or that H4K20me1 occurs following replication. Further investigation is required to elucidate the role of H4K20me1 in viral replication.

Collectively, Milavetz and colleagues have provided novel insights into how alterations of an expanding histone code within SV40 minichromosomes are likely to influence its function during lytic infection.

References

1. Strahl BD, et al. Nature 2000; 403:41-5.
2. Balakrishnan L, et al. Virology 2005; 334:111-23.
3. Rice JC, et al. Cell 2007; 129:915-28.
5. Schotta G, et al. Genes Dev 2004; 18:1251-62.
6. Martens JH, et al. EMBO J 2005; 24:800-12.
7. Pesavento JJ, et al. Genes Dev 2002; 16:2225-30.

©2010 Landes Bioscience. Do not distribute.

Virus-host interaction: The struggle for control of chromatin

Comment on: Balakrishnan L, et al. Cell Cycle 2010; 9:1320–32.

Ellen Fanning and Weston P. Dulaney; Vanderbilt University; Nashville, TN USA; Email: ellen.fanning@vanderbilt.edu

Animal viruses are exquisitely well adapted to re-direct the resources of their host cells to facilitate viral propagation. Viruses with a DNA genome that is expressed and replicated in the cell nucleus have evolved not only to contend with host enzymes that operate on chromatin templates, but also to exploit them for their own propagation or persistence in the host.[1,2]

Viruses of the polyoma family, which includes SV40 and the related human viruses BKV, JCV and MCV, contain a circular DNA of 5.2 kbp complexed with host histones 20–24 nucleosomes in a mini-chromosome.[3] SV40 enters the cell at the plasma membrane and moves through vesicular transport to the endoplasmic reticulum (ER), where ER oxidoreductases loosen the viral coat, exposing the minor capsid proteins VP2 and VP3.[4,5] The destabilized virus is ejected into the cytosol via ER-associated degradation ERAD) and the mini-chromosome enters the nucleus. There, host machinery transcribes, replicates, and packages daughter chromatin in a temporally regulated fashion under the direction of T antigen. Although the viral life cycle is known in some detail, our understanding of how SV40 manipulates the histone code to hijack cellular tools for its gene expression, replication, and virus packaging remains incomplete.

Early work established that purified SV40 particles contain hyperacetylated histones,[6] which, based on current knowledge, would facilitate their transcription and replication in the host.[7] More recently, Milavetz and colleagues used chromatin immunoprecipitation (ChIP) to map histone H3 and H4 hyperacetylation in SV40 chromatin as a function of time after infection. In this issue of *Cell Cycle* (Volume 9, issue 7), they extend this approach to analyze histone H4 methylation of lysine 20 (K20). They report three major new observations: (1) mono-methylated K20 (H4K20me1) is prominent in viral chromatin undergoing late transcription, replication and packaging into daughter virions, and in virus particles, but is reduced in viral chromatin during early transcription; (2) di-methylated H4K20 is not detectable in viral chromatin; and (3) tri-methylated K20 (H4K20me3) appears transiently in viral chromatin as the infecting virus makes its way into the cell. The appearance of H4K20me3 in viral chromatin at 0.5 hours post-infection (hpi) was followed by a drop in the total amount of intracellular viral DNA and the appearance of H4K20me3-containing fragments of viral chromatin by 4 hpi, suggesting that the tri-methylated fraction of the incoming viral chromatin undergoes degradation. These data imply a struggle between the virus and the host for control of

viral, and perhaps host, chromatin as the infection develops and raise intriguing new questions about the mechanisms involved.

One question is how H4K20me3, a modification typical of cellular heterochromatin,[7] is generated in viral chromatin at such early times in infection. Detailed characterization of the SV40 entry pathway demonstrates that virus particles require ~4 h to travel from the plasma membrane to the ER lumen, where the virus interior first becomes accessible; only at 5–6 hpi does viral chromatin reach the nucleus.[3,4,5] One possible resolution of this conundrum is that the infection is asynchronous, allowing detection of newly generated H4K20me3 at the 30-min time point reported here, but eliminating it before the 4-h time point. Another possibility is that H4K20me3 buried in the nucleosomes of the incoming virus becomes exposed upon ER-mediated destabilization of the capsid. It would be interesting to know whether ERAD-mediated virus exit to the cytosol[5] is correlated with either the appearance or destruction of H4K20me3-containing viral chromatin. Additional work will be needed to link the appearance and disappearance of H4K20me3 in viral chromatin to events and sites in the uncoating process.

A second intriguing question is whether SV40 infection may modulate the host milieu in a manner that inhibits additional methylation of H4K20me1 or precludes assembly of H4K20me2/me3 in viral nucleosomes. H4K20me2, but not the mono- or tri-methylated form, serves as a specific docking site in chromatin for the damage signaling/repair protein 53BP1.[8] Of note, Herpesvirus programs the proteolytic destruction of two host histone ubiquitin ligases whose activities are needed to expose nucleosomal H4K20me2 for stable 53BP1 docking.[2] Like Herpes, SV40 modulates cellular DNA damage signaling for successful viral propagation,[9] raising the possibility that it too may disrupt H4K20me2-linked damage signaling. If so, determining the mechanism may provide additional insight into the relationship between DNA damage signaling and repair.

References

1. Knipe DM, et al. Nat Rev Microbiol 2008; 3:211-21.
2. Lilley CE, et al. EMBO J 2010; 29: 943-55.
3. Neu U, et al. Virology 2009; 384:389-99.
4. Pelkmans L, et al. Nat Cell Biol 2001; 3:473-83.
5. Schelhaas M, et al. Cell 2007; 131: 516-29.
6. Vesco C, et al. Virology 1982; 118:389-400.
7. Campos EI, et al. Ann Rev Genet 2009; 41: 559-99.
8. Botuyan MV, et al. Cell 2006; 127:1361-73.
9. Zhao X, et al. J Virol 2008; 82:5316-28.
10. Balakrishnan L, et al. Cell Cycle 2010; 9:1320-32.

On the move: p27^{Kip1} drives cell motility in glioma cells

Comment on: See WL, et al. Cell Cycle 2010; 9:1562-7.

Bobby Bhatia; Memorial Sloan-Kettering Cancer Center; New York, NY USA; Email: bhatiab@mskcc.org

©2010 Landes Bioscience.
Do not distribute.

Cells from metastatic or invasive tumors require the ability to become motile and degrade the extracellular matrix and basement membranes, which are stabilized by actin stress fibers when producing tension across the cell, allowing the cell to pull and anchor on to a substrate (e.g., the extracellular matrix).[1] Actin stress fibers are stabilized by Rho GTPases that switch between inactive GDP-bound and active GTP-bound states.[2] Specifically, RhoA bound to GDP is activated by guanine–nucleotide exchange factors (GEFs). GTP-bound RhoA activates a signaling cascade leading to phosphorylation of cofilin, an actin-depolymerizing protein. This phosphorylation inhibits cofilin activity, resulting in stabilization of actin filaments. GTPase-activating proteins (GAPs) promote the transition of RhoA to the inactive (GDP-bound) state, releasing cofilin from inhibition and thereby leading to actin disassembly. Expression of the GAP domain of p190RhoGAP decreased the incidence of platelet derived growth factor (PDGF)-induced gliomas in mice.[3] Furthermore, more than half of human oligodendrogliomas contain deletions in chromosome 19q13.3, a region that contains p190RhoGAP4. These observations suggest that Rho activity is significant for gliomagenesis.

The cell cycle regulator p27^{Kip1} plays a critical role in cell cycle progression at the G_1-S phase transition by binding to and inactivating cyclin E-Cdk25. p27^{Kip1} also regulates cell cycle progression by facilitating the assembly of cyclin D1-Cdk4. In human oligodendrogliomas, loss of p27^{Kip1} correlates with poor prognosis and decreased survival6. Interestingly, the status of p27^{Kip1} does not correlate with proliferation, suggesting a potential cell cycle-independent function. Recent reports indicate that mislocalized p27^{Kip1} increases cell migration by potentially inhibiting activation of RhoA,[7,8] but the mechanisms by which p27^{Kip1} regulates this process remain unclear.

In an exciting study by See et al., (*Cell Cycle*, Volume 9, Issue 8) p27^{Kip1} affects migration of glioma cells via regulation of Rho. Using a retrovirus-mediated delivery system (RCAS/tv-a) to induce gliomagenesis by constitutively overexpressing PDGF in nestin-positive progenitor cells in the brain,[9,10] the authors find that p27^{Kip1} deficiency leads to elevated Rho-GTP levels in PDGF-driven glioma cells derived from p27^{Kip1} knockout mice compared to wild type mice. But is the relationship between p27^{Kip1} and Rho activity vital for glial cell growth and migration? To determine this, the authors treated these cells with Rho kinase inhibitor Y27632 leading to a slower growth rate. Interestingly, cofilin phosphorylation was higher in p27^{Kip1} knockout cells compared to wild type cells, suggesting the knockout cells are less sensitive to Rho kinase inhibition due to elevated Rho-GTP levels.

To further strengthen the hypothesis, See et al. performed invasion assays and observed that wild type cells migrated at a three-fold higher rate compared to p27^{Kip1} knockout cells. Y27632 treatment in p27^{Kip1} knockout cells rescued the invasion defect and was equivalent to untreated wild type cells. However, Y27632 treated knockout cells could not reach migration levels in Y27632 treated wild type cells, suggesting again p27^{Kip1} deficient cells are less migratory due to enhanced Rho activation. Conversely, See et al. found that exogenous p27^{Kip1} expression increased migration of p27^{Kip1} knockout cells in a wound-healing assay using wild type p27^{Kip1} or strikingly p27-CK-, a mutant of p27 that cannot bind cyclins/cdks, suggesting this may occur in a cell cycle independent manner.

To date, it has been unclear whether loss of p27^{Kip1} regulates migration of tumor cells in vivo. See et al. observed that survival latency was dramatically decreased in p27^{Kip1} deficient mice, and this was correlated

with hydrocephaly. These mice had low grade tumors localized in the lateral ventricles, failing to invade the normal brain parenchyma, whereas wild type tumors include parenchymal invasion, indicating that p27^{Kip1} deficient PDGF-expressing gliomas are less invasive in vivo. However, is this a coincidence or does these tumors really depend on Rho and p27^{Kip1} for cell migration in vivo? To gain mechanistic insight, See et al. co-expressed PDGF with p27^{Kip1}, p27-CK-, or the GAP domain of p190RhoGAP, in p27^{Kip1} deficient mice and found a dramatic decline of ventricular tumors that correlated with reduced hydrocephaly. Strikingly, this led to higher parenchymal invasion incidence and longer survival. It is unknown in this study whether the extended survival eventually leads to tumorigenesis, however the data presented here adds weight to the cell cycle-independent role of p27^{Kip1}. Although the authors did not state whether p27^{Kip1} regulates Rho directly or indirectly, they do provide strong evidence that glioma tumor cells depend on the relationship of p27^{Kip1} and Rho for cell migration and invasion. More importantly, there is now insight on the double-edged sword nature of p27^{Kip1} that may be a focus of future glioma therapy.

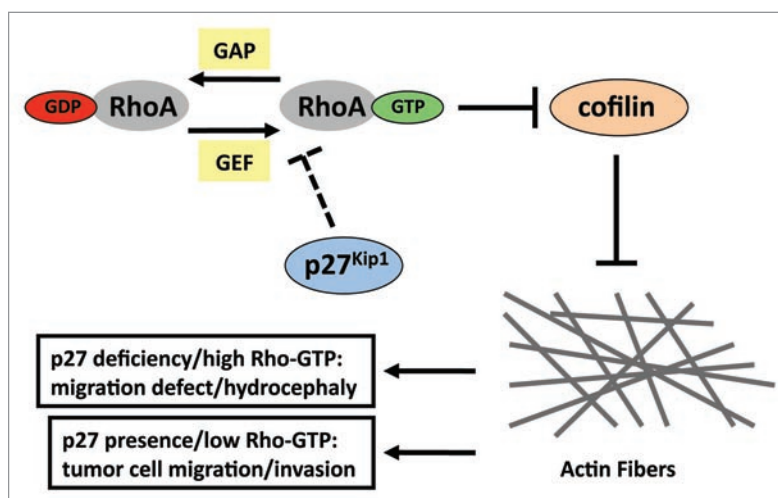

Figure 1. Inhibition of the Rho pathway by p27^{Kip1} leads to disassembly of the actin stress fiber filaments and promotion of cell migration/invasion. Rho GTPases switch between inactive GDP-bound and active GTP-bound states. GTPase-activating proteins (GAPs) promote the inactive state whereas guanine–nucleotide exchange factors (GEFs) promote the active state. In p27^{Kip1} deficiency, RhoA is activated, and then GTP-bound RhoA activates ROCK to phosphorylate its substrate LIMK (not shown). This enhances the ability of LIMKs to phosphorylate and inactivate cofilin, an actin-depolymerizing protein (not shown). Phosphorylation of LIMKs by ROCKs prevents reorganization of actin filaments and thereby leads to an increase in stress fibers. However, in the presence of p27^{Kip1}, RhoA is inactive, leading cofilin to disassemble actin filaments, which in turn increases cell migration/invasion.

©2010 Landes Bioscience.
Do not distribute.

References

1. Hall A. Cancer Metastasis Rev 2009; 28:5-14.
2. Schmidt A, et al. Genes Dev 2002; 16:1587-609.
3. Wolf RM, et al. Genes Dev 2003; 17:476-87.
4. Tikoo A, et al. Gene 2000; 257:23-31.
5. Larrea MD, et al. Cell Cycle 2009; 8:3455-61.
6. Cavalla P, et al. Acta Neuropathol 1999; 98:629-34.
7. Besson A, et al. Genes Dev 2004; 18:862-76.
8. Larrea MD, et al. Proc Natl Acad Sci USA 2009; 106:9268-73.
9. See WL, et al. Oncogene 2010; 29:1720-31.
10. See WL, et al. Cell Cycle 2010; 9: 1562-7.

Evidences of cervical cancer stem cells derived from established cell lines

Comment on: Bortolomai I, et al. Cell Cycle 2010; 9:1194–1206.

Matilde Todaro,[1] Ylenia Lombardo[1,2] and Giorgio Stassi[1,2]; [1]University of Palermo; Palermo, Italy; [2]IRCCS Fondazione Salvatore Maugeri; Pavia, Italy;
Email: giorgio.stassi@unipa.it

According to the longstanding "clonal evolution" model of carcinogenesis, cervical carcinoma has long been described as a consequence of unlimited and uncontrolled cellular proliferation conferred by multiple genetic and/or epigenetic mutations that can hit any somatic cells within the tissue. However, in the last few years, accumulating evidence has suggested that the capacity of initiating a tumor, including cervical carcinoma, is rather a unique feature of a small subset of stem-like cells called "cancer stem cells" (CSCs) or "tumor-initiating cells." CSCs have the exclusive ability to self-renew expanding the CSCs pool, and to maintain the tumor differentiating into the heterogeneous non tumorigenic cancer cell types which constitute the majority within the tumor.[1]

Although solid evidence is lacking to date, subcolumnar reserve cells emerged to be the best candidate for cervical stem cells, which provide a depository for the regeneration of the mucous-forming epithelium. Such long-lived stem cells are potential targets for malignant transformation playing the leading role in the development of cervical carcinoma.[2]

The CSCs hypothesis has exciting clinical implications in cervical cancer: it could explain therapy failure and relapses as the result of CSCs resistance to death stimuli. Indeed, retaining the biological hallmarks of tissue stem cells, such as quiescence, self-renewal ability and multidrug resistance, CSCs constitute the population intrinsically refractoriness to therapies developed to eradicate the rapidly dividing cells. Therefore, identification and characterization of CSCs is fundamental for prognosis and treatment of cervical carcinoma.

Nanog, nucleostemin and musashi1 have been identified as the first putative stem cell markers in human cervical carcinoma.[3] A recent report used the CD44 and CD17 cell-surface markers to enrich cancer stem-like cell population. Further investigation revealed that this cellular pool possesses tumorigenic capacity and expresses embryonic and adult stemness-related genes (Oct-4, Piwil2, C-myc, Stat3 and Sox2).[4]

In the last issue of *Cell Cycle*, Bortolomai et al. isolated cervical stem cells from the A431cell line exploiting the capability of these cells to form spheres in non-adherent conditions. Following isolation, ALDH enzymatic activity and Hoechst dye exclusion were employed to further confirm the presence and size of the stem-like population. Importantly, although A431 sphere cells were characterized by "stemness" properties such as self-renewal and clone forming capability, in adherent conditions they mainly formed differentiated colonies (paraclones) compared to A431 parental cells. These data, in spite of the apparent contradiction with the assumption that loss of anchorage and serum deprivation induce *anoikis* of differentiated cells,[5] could underlay the multipotent differentiative potential of sphere cells. Indeed, A431 sphere cells could be considered an experimental strategy to select and expand in suspension "holoclones" from a heterogenous population of cancer cells. It thus became clear that in culture conditions promoting differention, sphere cells gave rise to more differentiated colonies.

Long-term cultures in suspension confirmed that sphere cells maintain an higher EGFR stimulation-independent proliferative potential than parental cells. Moreover, A431 spheres display high expression levels of self-renewal-related genes such as NANOG, NESTIN and OCT4, and, more importantly, an enrichment of podoplanin-positive cells, which were recently described as the stem-like population of A431 cells.

Cancer stem cell nature of A431 spheres was thus definitely proved by their ability to recapitulate the generation of a continuously growing tumor, which display a more undifferentiated morphology than original human tumor phenotype from the fourth passage into immunocompromised mice.

Finally, the global gene expression profile of A431 sphere cells analyzed in comparison with that of A431 parental and adherent cells rederived from A431 spheroids, revealed genes encoding for Cytokeratin 6 and Osteopontin, were highly upregulated.

From this study, Osteopontin emerges as a good stem cell marker for cervical cancer. Through its interaction with integrins and CD44v, osteopontin is known to induce metalloprotease, thus representing an important modulator of cell motility.[6] Osteopontin supports the hypothesis that cells with stem-like properties within the neoplastic mass are responsible, apart from tumor initiation, also for invasion of surrounding tissues and metastasis. If invasive and metastatic properties are typical features of these cells, the newly developed salinomycin compound, could be efficient to eradicate cervical cancer.[7] This agent regulates the migration inhibiting potassium channels and consequently interfering with epithelial-mesenchymal transition.[8]

Together with the identification of the phenotypic characteristics of cervical cancer stem cells examined by Bortolomai and coworkers a better understanding of metastatic mechanisms intrinsic to CSCs will provide essential tools for prediction and successful treatment of this malignant tumor.

Acknowledgements

The authors are supported by AIRC.

References

1. Visvader JE, et al. Nat Rev Cancer 2008; 8:755-68.
2. Martens JE, et al. Int J Gynecol Pathol 2007; 26:463-8.
3. Ye F, et al. BMC Cancer 2008; 8:108.
4. Feng D, et al. Oncol Rep 2009; 22:1129-34.
5. Charafe-Jauffret E, et al. Pathobiology 2008; 75:75-84.
6. Rangaswami H, et al. Trends Cell Biol 2006; 16:79-87.
7. Gupta PB, et al. Cell 2009; 138:645-59.
8. Lin LF, et al. Cancer Res; 70:192-201.

©2010 Landes Bioscience.
Do not distribute.

From Dolly to hiPS: New insights into reprogramming

Comment on: Lagarkova MA, et al. Cell Cycle 2010; 9:937-46.

Jürgen Hescheler; University of Cologne; Cologne, Germany; Email: j.hescheler@uni-koeln.de

When Ian Wilmut presented in 1997 his remarkable result that a somatic nucleus could be reprogrammed when transferred into the cytosolic environment of an enucleated oocyte (SCNT, somatic cell nuclear transfer) giving rise to a full mammalian offspring (the clone sheep Dolly)[1] the new area of inducing pluripotency has been born. It is interesting that it took more than a decade to further investigate the basis of this observation. Nowadays we learn more and more on mechanisms and since the notable work of Shinya Yamanaka and colleagues[2,3,4] we can minimize the necessary factors to four transcription factors or even less.[5] A recent issue of *Cell Cycle* goes along with this development and presents the article by Maria A. Lagarkova and coworkers: "Induction of pluripotency in human endothelial cells resets epigenetic profile on genome scale." Two new aspects are demonstrated: The team around Prof. Kiselev found that induced pluripotent cells (iPS cells) can be generated from human umbilical vein endothelial cells (HUVEC) by the conventional method of overexpressing oct4, sox-2, c-myc and klf4 and secondly using a full characterization of their functional and epigenetic properties they clearly demonstrate that iPS cells generated from fully differentiated somatic cells reset their epigenetic status to pluripotency. From this as well as from other studies it becomes now quite evident that the clue of reprogramming lies in epigenetic mechanisms establishing cellular identity during differentiation by (1) DNA cytosine methylation, (2) covalent histone modifications (3) remodeling of other chromatin associated proteins such as polycomb group proteins and transcription factors, and (4) pre- and post-transcriptional gene regulation by small non-coding RNAs, such as microRNAs.[6] During epigenetic reprogramming of the somatic nucleus, these modifications, in particular DNA methylation must be reset from a fully differentiated to a pluripotent state. The work by Lagarkova et al. succeeded to obtain human iPS cells from human endothelial cell (endo-iPS) which were similar to human embryonic stem cells in morphology and gene expression. Using genome-wide methylation profiling the group shows in particular that the promoter elements of endothelial specific genes were methylated following reprogramming. On the other side, the pluripotency-related promoters were hypomethylated to levels also observed in embryonic stem cells. A similarity between endo-iPS and ES cells was seen in the genome-wide methylation analysis of CpG sites located in the functional regions of over than 14,000 genes but it is interesting to mention that the methylation levels of 46 genes were found to

be different suggesting that these sites represent endothelial genes which are resistant to reprogramming. Overall CpG methylation of promoter regions in the pluripotent cells was higher than in somatic. Within their work, the team around Kiselev also demonstrated that during reprogramming female human endo-iPS cells the somatically silenced X chromosome are obviously reactivated leading to the notion that chromosomal inactivation is epigenetically regulated and thus reversible. All these data now strongly give us the aim to reach more understanding on the underlying mechanism occurring after overexpression of the "Yamanaka-factors." Why does the before thought irreversible epigenetic inactivation of the female X chromosome comes out to be reversible while other genes obviously are not reverted? Are there additional mechanisms controlling the inactivation of genes which are not subject to reprogramming? Is this a limitation of the iPS-strategy for a later clinical usage of these cells? Is the function affected as recently demonstrated in electrophysiological studies?[7] Do we need more sufficient reprogramming techniques based on a deeper insight into the genetic programming and reprogramming? How does this relate to possible dedifferentiation and carcinogenesis? After Dolly, which obviously resulted from a complete reprogramming of a somatic cell into the pluripotency, we have reached a lot but we still need more experimental data to get insights into processes of epigenetic memory and genetic regulation of the cell fate to a specific phenotype.

References

1. Wilmut I, et al Nature 1997; 6619:810-13.
2. Takahashi K, et al. Cell 2006; 126:663-76.
3. Okita K, et al. Nature 2007; 448:313-7.
4. Nakagawa M, et al. Nat Biotechnol 2007; 26:101-6.
5. Kim JB, et al. Nature 2009; 461:649-53.
6. Wu H, et al. Pediatr Res 2006; 59:21R–5R.
7. Kuzmenkin A, et al. FASEB J 2009; 23:4168-80.

©2010 Landes Bioscience.
Do not distribute.