JB Review

J. Biochem. **141,** 453–458 (2007)
doi:10.1093/jb/mvm059

Regulation of Cellular Transformation by Oncogenic and Normal Abl Kinases

Jun Suzuki[1,2] and Tomoyuki Shishido[1,*]

[1]Laboratory of Molecular Oncology, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 630-0192; and [2]Graduate School of Medicine, Osaka University, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan

Received September 1, 2006; accepted February 9, 2007; published online February 16, 2007

Cellular transformation, the conversion of normal cells into tumorigenic cells *in vitro*, is characterized by immortalization, anchorage- and serum-independent growth and tumour formation in the nude mouse. Among these, anchorage-independent growth is one of the defining characteristics of transformed cells and tumour cells. Without attachment to the extracellular substrate, most normal cells cannot grow or survive, but tumour cells can proliferate. Many oncogenes and tumour suppressors are involved in regulating this process, among which is Abl tyrosine kinases. Previous work showed that v-Abl, an oncogenic variant of c-Abl kinase, induces anchorage-independent growth in the context of p53 deficiency, and a recent study by our group showed that loss of c-Abl kinase also facilitates anchorage-independent growth. The cellular context, such as a deficiency in both p53 and RB, is critical to induce anchorage independence by loss of c-Abl kinase. In this review, we discuss the mechanisms of cellular transformation by oncogenic and normal Abl kinases.

Key words: Abl kinases, anchorage independence, Bcr-Abl, c-Abl, cellular transformation, v-Abl.

Abbreviations: ECM, Extracellular matrix; MFFs, mouse embryonic fibroblasts; CDK, Cylcin-dependent kinase; PR, Proline rich; ALV, Abelson leukemia virus; CML, chronic myelogenous leukemia.

How do normal cells become tumorigenic? This remains one of the most fundamental questions in the field of cancer science. Although *in vivo* mouse models (knockout or transgenic) gives robust evidence that certain genes are involved in tumour development, the precise mechanism(s) also need to be examined at the cellular level, because tumorigenesis is a process accompanied by multiple genetic and epigenetic alterations in cells. Cellular transformation involves the conversion of normal cells into malignant cells *in vitro*, and the transformed cells acquire distinctive features such as immortalization, anchorage-independent growth, serum-independent growth and tumour formation in the nude mouse. Among these features, anchorage-independent growth capability *(in vitro*) correlates well with tumour formation in the nude mouse (*in vivo*) (*1–3*), and is therefore a prominent characteristic to define cellular transformation (*4*). Without attachment to the extracellular matrix (ECM), normal cells cannot grow or survive, but tumour cells can proliferate. This indicates that adhesion-dependent integrin signalling from the ECM determines the growth properties of cells stimulated with growth factors (*5–7*). Remarkably, growth factor-mediated and integrin-mediated signalling regulate the same signalling pathways (*8*). Efficient activation of the Ras/Raf/MAPK pathway or the PI3K/Akt pathway by growth factor requires cell adhesion (*9–11*). Cell adhesion also regulates expression of c-fos and c-myc (*12*), which is controlled by the Ras/Raf pathway (*13*, *14*). Considering that constitutive activation of Ras, Raf and Mek facilitates anchorage- and serum-independent growth of established cell lines, it is likely that regulation of the Ras/Raf/Mek pathway is invariably important in the process of tumorigenesis. Activation of this signalling pathway induces cyclin D1 expression, which regulates the G1/S transition (*15*). Integrins and growth factors cooperatively regulate cyclin D1 expression via cytoplasmic signalling pathways (*16*). Knockout of all D-type cyclins (D1, D2 and D3) showed that cyclin D is a critical mediator of oncogenic Ras-induced transformation of mouse embryonic fibroblasts (MEFs) (*17*). Although cyclin D1 regulates the cell cycle in cyclin-dependent kinase (CDK)-dependent and -independent manners (*18*, *19*), it remains to be elucidated how this dual regulation contributes to the process of cellular transformation.

It is noteworthy that expression of activated Ras in primary MEFs provokes irreversible growth arrest, known as premature senescence, via p53 or $p16^{ink4a}$ (*20*), indicating that a single oncogene is insufficient to induce malignant cellular transformation. How, then, are oncogenes involved in transformation? Outstanding work in the 1980s revealed that a combination of oncogenes converts primary rodent cells into tumorigenic cells: oncogenic Ras cooperates with adenovirus E1A, v-myc or SV40 T antigen to transform primary rodent cells (*21–23*), suggesting that cytoplasmic and nuclear oncoproteins collaborate in malignant transformation

*To whom correspondence should be addressed. Tel: +81-743-72-5541, Fax: +81-743-72-5549, E-mail: shishid@bs.naist.jp

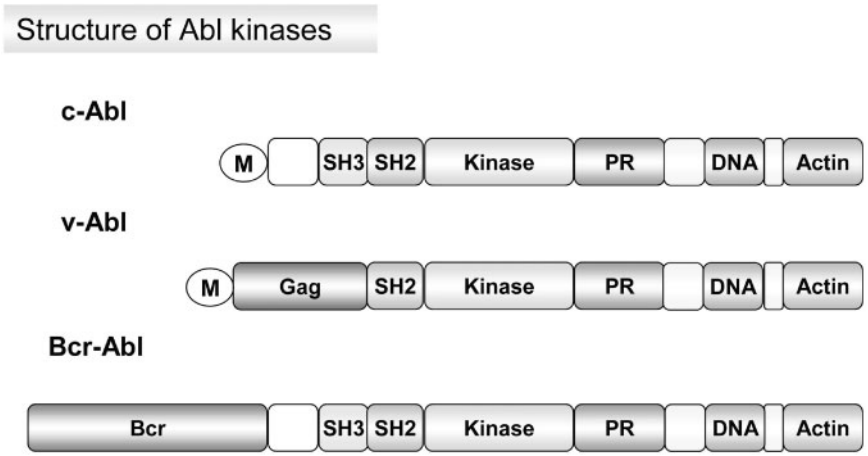

Fig. 1. **Structure of Abl kinases.** c-Abl contains a myristoylation modification signal (M), SH3 domain, SH2 domain, kinase domain, proline rich region (PR), DNA-binding domain, and actin-binding domain. An SH3 domain in v-Abl is replaced with the viral gene *gag*. In Bcr-Abl, a *Bcr* gene is fused to the N terminus of c-Abl, resulting in loss of the myrstoylation modification signal.

(*24*). In contrast to rodent cells, human cells are known to be more refractory to oncogenic transformation (*25*). However, Hahn *et al*. (*26*) showed that introduction of the catalytic subunit of telomerase (hTERT) facilitates transformation of primary human cells in combination with activated Ras and SV40 T antigen. This combination, however, converts human cells into full malignant transformants only weakly (*27–29*). Akagi and colleagues showed that Ras-induced activation of Erk is attenuated in human cells but not in rodent cells. This, in turn, abrogates up-regulation of Fra1 or down-regulation of Caveolin-1, both of which are required for anchorage-independent growth of human cells (*30*, *31*). These observations emphasize that differences between model systems (*e.g*., human or rodent) should always be kept in mind. Although combining oncogenes successfully convert normal cells into tumorigenic cells in terms of anchorage-independent growth, the precise mechanisms need to be further investigated.

Regulation of Cellular Transformation by Abl Kinases—c-abl is the proto-oncogene of the Abelson leukaemia virus (ALV) oncogene *v-abl* and encodes a non-receptor tyrosine kinase (*32*, *33*). Structural differences between c-Abl and v-Abl are shown in Fig. 1. The N-terminal region of c-Abl contains a myristoylation modification signal, an SH3 domain, and an SH2 domain. The SH3 domain is replaced with the viral protein Gag in v-Abl, which results in acquisition of hyper tyrosine kinase activity. In Bcr-Abl, Bcr is fused to the N-terminus of c-Abl, and the coiled-coil region of Bcr contributes to dimerization between Bcr-Abl molecules, resulting in induction of up-regulated kinase activity.

Although ALV transforms pre-B cells in mice, and v-Abl is required for this function, it primarily induces apoptosis in B cells (*34*). In p53 or $p19^{Arf}$ knockout mice, however, ALV fails to induce apoptosis and efficiently transforms pre-B cells (*35*, *36*). Although it is unclear whether inactivation of the $p19^{Arf}$/p53 pathway and expression of v-Abl is enough to convert normal pre-B cells into transformed cells, these studies suggest that v-Abl transforms cells depending on the cellular context, as does activated Ras. In primary MEFs, expression of v-Abl induces senescence, but inactivation of the p53/$p19^{Arf}$ pathway renders the cells susceptible to transformation by v-Abl (*37*). Expression of v-Abl is required for both initiation and maintenance of the transformed phenotype, because treatment with the Abl inhibitor STI571 (Gleevec/Imatinib) stops proliferation of transformed-pre-B cell lines (*38*). In the immortalized murine cell line NIH3T3, v-Abl facilitates anchorage- and serum-independent growth or growth arrest at the G1 phase, depending on the cellular context (*39*). Overexpression of c-Abl in NIH3T3 cells also facilitates growth arrest at the G1 phase but rarely facilitates transformation (*40*). NIH3T3 cells are null for both $p16^{ink4a}$ and $p19^{Arf}$, but express wild-type p53 (*41*). Although the precise mechanism is unknown, c-Abl induces growth arrest rather than senescence or apoptosis depending on the cellular context, and at least $p16^{ink4a}$ and $p19^{Arf}$ are dispensable for this function.

From the above observation, it appears that Abl kinases generate both positive and negative signals for growth, and that disabling the negative signal facilitates oncogenic transformation. The mechanisms can be explained by differences between the effects on Abl kinase substrates of both positive and negative signals. Because kinase activity of c-Abl is autoinhibited by intramolecular folding of the N-terminus onto the kinase domain (*42*, *43*), replacement of SH3 domain with the viral protein Gag may facilitate disruption of autoinhibited kainse activity, as occurs with Bcr-Abl (*44*). Thus, the balance between positive and negative signals on substrate phosphorylation is altered in v-Abl-transformed cells compared to normal cells expressing c-Abl. It is known that an Abl-binding protein, Abi-1, suppresses transformation of v-Abl-expressing NIH3T3 cells (*45*, *46*). We have found that Abi-1 binds to both c-Abl and substrates and works as a regulator of both kinase and substrate (*47–49*), and others have found novel c-Abl substrates that are phosphorylated

by this mechanism (*50–52*). These observations suggest that the negative signal for growth by Abl comprises signalling pathways of Abi-1-mediated substrates of Abl. Moreover, overexpression of c-Cbl, a substrate of Abl (*53*, *54*), in v-Abl expressing NIH3T3 cells inhibits anchorage-independent growth (*55*). Overexpression of DokL, a binding partner of Abl, also suppresses v-Abl-mediated transformation of NIH3T3 cells (*56*). Collectively, these observations suggest that Abl constitutes a negative signal via Abi-1, c-Cbl, DokL, and possibly other proteins.

In contrast to Abi-1 and c-Cbl, overexpression of c-Crk, a regulator and substrate of c-Abl (*57*, *58*), facilitates anchorage-independent growth of NIH3T3 cells via activation of focal adhesion kinase (FAK) by making a complex with Cas^{p130} (*59*). Because phosphorylation of c-Crk by Abl promotes disassembly of the Crk/Cas^{p130} complex and inhibits anchorage-independent growth (*60*), it is more likely that overexpression of c-Crk overcomes c-Abl-mediated disruption of the Crk/Cas^{p130} complex. In breast cancer cell lines, c-Crk phosphorylation by c-Abl downstream of the EphB4 receptor inhibits viability, proliferation, motility and invasion, suggesting that the c-Abl-c-Crk pathway negatively regulates transformation (*61*). It is still unknown whether overexpression of c-Crk in v-Abl-transformed cells constitutes a positive or negative signal for anchorage independence.

We have shown that the loss of c-Abl, and specifically loss of its kinase activity, also facilitates anchorage-independent growth of MEFs in the context of p53- and RB-deficiency (*62*). In adherent cells, the phosphorylation status of MAPK and Akt is not significantly different in wild-type and *c-abl*-knockout MEFs, but *c-abl*-knockout MEFs show enhanced phosphorylation of these signals when compared to wild-type MEFs in suspension (J. Suzuki and T. Shishido, unpublished data). It is noteworthy that c-Abl promotes proliferation of MEFs lacking p53 in the adherent state (*63*). Taken together, these observations suggest that normal levels of endogenous c-Abl are required to promote adherent growth and inhibit anchorage-independent growth in the context of p53 deficiency.

How is anchorage independence regulated positively by v-Abl and negatively by c-Abl? One possibility is that oncogenic variants of Abl eliminate negative signals for anchorage-independent growth normally generated by Abl. This model of anchorage-independent growth by Abl is shown in Fig. 2. One candidate downstream protein is Caveolin-1, which was identified as a substrate of v-Src. In v-Src-, RasV12- or v-Abl-transformed MEFs, expression of Caveolin-1 decreases due to MAPK-dependent and -independent down-regulation of gene expression (*64*). Expression of Caveolin-1 in v-Abl- or RasV12-transformed NIH3T3 cells suppresses anchorage-independent growth (*65*). Caveolin-1 is strongly phosphorylated at tyrosine 14 in v-Abl-expressing cells (*66*, *67*). Although, it remains to be elucidated whether c-Abl directly phosphorylates Caveolin-1, oxidative stress-induced tyrosine phosphorylation of Caveolin-1 does not occur in *c-abl*-knockout MEFs (*68*). Phosphorylation of Caveolin-1 tyrosine 14 has been implicated in negative regulation of anchorage-independent growth via inhibition of Erk, PI3K and

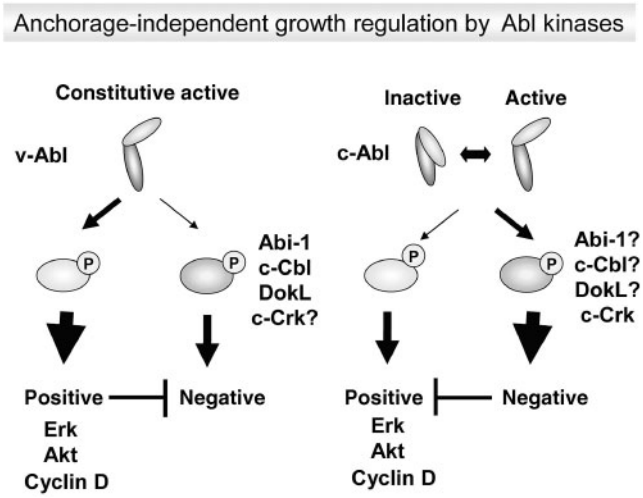

Fig. 2. **Regulation of anchorage-independent growth by Abl kinases.** An oncogenic variant of Abl, v-Abl possesses constitutive tyrosine kinase activity. Although Abl phosphorylates substrates which constitute positive and negative signals for growth, v-Abl is likely to output more positive signal, which, in turn, inhibits the negative signal generated by v-Abl itself. Positive signal by Abl comprises of activation of Erk, Akt and cyclin D, but it is unclear which substrates of Abl regulate this pathway. Substrates of Abl regulating negative signal for growth is likely to be Abi-1, c-Cbl, DokL and c-Crk. Unlike v-Abl, kinase activity of c-Abl is tightly regulated and fluctuates between inactive and active states. c-Abl outputs more negative signal for growth in suspension, which blocks proliferation.

Rac by enriched membrane microdomain internalization (*69*). In summary, c-Abl may partly suppress anchorage-independent growth by phosphorylating Caveolin-1, an effect which may be abrogated in v-Abl-expressing cells by transcriptional repression of *Caveolin-1* gene expression (Fig. 3). However, it remains to be determined which substrates of c-Abl are indeed involved in inhibition of positive signals regulating anchorage-independent growth.

Regulation of Malignant Transformation in vivo by Abl Kinases—Although a recent paper showed that c-Abl is constitutively activated in some breast cancer cell lines, and that its activity is required for invasion (*70*), it is unclear whether c-Abl is involved in development of solid tumours. Because *c-abl*-knockout mice die soon after birth, it is currently unknown whether c-Abl works as a tumour suppressor *in vivo*. In contrast, oncogenic variants of Abl (Bcr-Abl, Tel-Abl and v-Abl) induce leukaemia. Expression of v-Abl causes pre-B cell leukaemia in mice and sarcoma in cats. Bcr-Abl, which is generated by chromosomal translocation, causes chronic myelogenous leukaemia (CML) (*71*, *72*) or acute lymphoblastic leukaemia in humans (*73–76*). CML evolves from a chronic phase to an accelerated phase, and finally to blast crisis, which is characterized by the rapid expansion of a population of myeloid or lymphoid differentiation-arrested blast cells (*77*). The mechanisms of transition into blast crisis are largely unknown. We found that expression of endogenous c-Abl is lost in several CML cell lines, established from blast crisis patients (*62*). Asimakopoulos *et al*. (*78*) have shown that

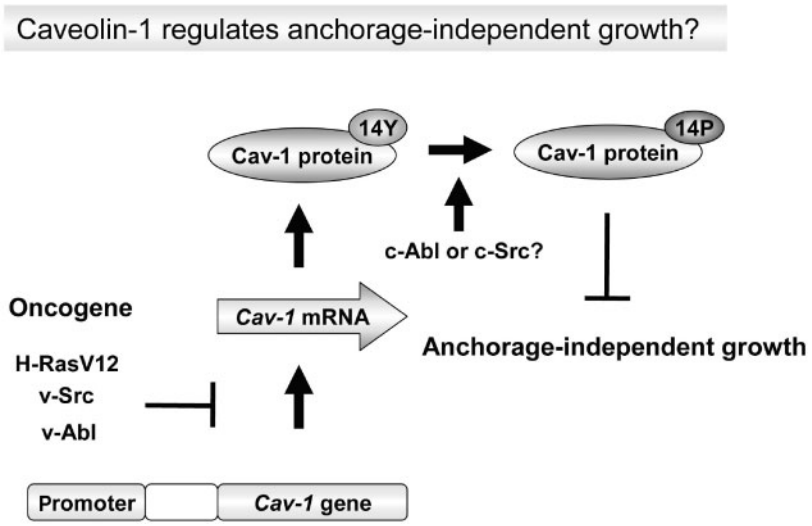

Fig. 3. **Regulation of anchorage-independent growth by Caveolin-1.** Expression of *Caveolin-1* is repressed by oncogenes such as H-RasV12, v-Src and v-Abl at the transcriptional level. Caveolin-1 is a candidate downstream target of Abl and Src family kinases. Phosphorylation (P) of Caveolin-1 on tyrosine (Y) 14 negatively regulates anchorage-independent growth, but it remains to be elucidated which kinases are involved in this regulation.

the regulatory regions of *c-abl* are hypermethylated in CML-blast crisis cells. These observations imply that loss of c-Abl expression is associated with transition into blast crisis. Because Bcr-Abl fails to induce anchorage-independent growth in NIH3T3 cells, unlike v-Abl (*79*, *80*), it is possible that loss of *c-abl* expression in the Bcr-Abl-expressing cells facilitates anchorage-independent growth as observed in p53- and RB-deficient MEFs (*62*). Whether this scenario is applicable to leucocytes is currently unknown. Because blast crisis is characterized by accumulation of myeloid or lymphoid blast cells, it needs to be determined whether loss of c-Abl in Bcr-Abl-expressing cells arrests differentiation.

Conclusions and Future Perspectives—Cellular transformation of primary cells is a powerful system to investigate the role of target genes in tumorigenesis. Using this system, the involvement of Abl kinase in anchorage-independent growth was revealed. c-Abl functions uniquely to induce or suppress anchorage-independent growth in a cellular context-dependent manner. A priority for future study is to identify the main target of c-Abl in negative regulation of anchorage independence. This will help us to understand how v-Abl escapes from the negative signals produced by v-Abl itself. Furthermore, it will be interesting to establish whether c-Abl is deregulated in Bcr-Abl-expressing cells, especially those cells in the blast crisis phase.

We thank Ian Smith for critically reading the manuscript. We acknowledge Hidesaburo Hanafusa and Tatsuo Takeya for support and discussion. This work was supported by Grants-in-Aid (16770128 and 18770172) and Aid for 21st Century COE Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan. J. S. was supported by a JSPS Research Fellowships for Young Scientists.

REFERENCES

1. Freedman, V.H. and Shin, S.I. (1974) Cellular tumorigenicity in nude mice: correlation with cell growth in semi-solid medium. *Cell* **3**, 355–359
2. Shin, S.I., Freedman, V.H., Risser, R., and Pollack, R. (1975) Tumorigenicity of virus-transformed cells in nude mice is correlated specifically with anchorage independent growth in vitro. *Proc. Natl. Acad. Sci. USA* **72**, 4435–4439
3. Gallimore, P.H., McDougall, J.K., and Chen, L.B. (1977) In vitro traits of adenovirus-transformed cell lines and their relevance to tumorigenicity in nude mice. *Cell* **10**, 669–678
4. Jove, R. and Hanafusa, H. (1987) Cell transformation by the viral src oncogene. *Annu. Rev. Cell Biol.* **3**, 31–56
5. Schwartz, M.A., Schaller, M.D., and Ginsberg, M.H. (1995) Integrins: emerging paradigms of signal transduction. *Annu. Rev. Cell Dev. Biol.* **11**, 549–599
6. Yamada, K.M. and Miyamoto, S. (1995) Integrin transmembrane signaling and cytoskeletal control. *Curr. Opin. Cell Biol.* **7**, 681–689
7. Assoian, R.K. (1997) Anchorage-dependent cell cycle progression. *J. Cell Biol.* **136**, 1–4
8. Schwartz, M.A. (1997) Integrins, oncogenes, and anchorage independence. *J. Cell Biol.* **139**, 575–578
9. Lin, T.H., Chen, Q., Howe, A., and Juliano, R.L. (1997) Cell anchorage permits efficient signal transduction between ras and its downstream kinases. *J. Biol. Chem.* **272**, 8849–8852
10. Renshaw, M.W., Ren, X.D., and Schwartz, M.A. (1997) Growth factor activation of MAP kinase requires cell adhesion. *EMBO J.* **16**, 5592–5599
11. Khwaja, A., Rodriguez-Viciana, P., Wennstrom, S., Warne, P.H., and Downward, J. (1997) Matrix adhesion and ras transformation both activate a phosphoinositide 3-OH kinase and protein kinase B/Akt cellular survival pathway. *EMBO J.* **16**, 2783–2793
12. Dike, L.E. and Farmer, S.R. (1988) Cell adhesion induces expression of growth-associated genes in suspension-arrested fibroblasts. *Proc. Natl. Acad. Sci. USA* **85**, 6792–6796
13. Eferl, R. and Wagner, E.F. (2003) AP-1: a double-edged sword in tumorigenesis. *Nat. Rev. Cancer* **3**, 859–868

14. Adhikary, S. and Eilers, M. (2005) Transcriptional regulation and transformation by Myc proteins. *Nat. Rev. Mol. Cell Biol.* **6**, 635–645
15. Roovers, K. and Assoian, R.K. (2000) Integrating the MAP kinase signal into the G1 phase cell cycle machinery. *Bioessays* **22**, 818–826
16. Schwartz, M.A. and Assoian, R.K. (2001) Integrins and cell proliferation: regulation of cyclin-dependent kinases via cytoplasmic signaling pathways. *J. Cell Sci.* **114**, 2553–2560
17. Kozar, K., Ciemerych, M.A., Rebel, V.I., Shigematsu, H., Zagozdzon, A., Sicinska, E., Geng, Y., Yu, Q., Bhattacharya, S., Bronson, R.T., Akashi, K., and Sicinski, P. (2004) Mouse development and cell proliferation in the absence of D-cyclins. *Cell* **118**, 477–491
18. Sherr, C.J. and Roberts, J.M. (1999) CDK inhibitors: positive and negative regulators of G1-phase progression. *Genes Dev.* **13**, 1501–1512
19. Ewen, M.E. and Lamb, J. (2004) The activities of cyclin D1 that drive tumorigenesis. *Trends Mol. Med.* **10**, 158–162
20. Serrano, M., Lin, A.W., McCurrach, M.E., Beach, D., and Lowe, S.W. (1997) Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. *Cell* **88**, 593–602
21. Ruley, H.E. (1983) Adenovirus early region 1A enables viral and cellular transforming genes to transform primary cells in culture. *Nature* **304**, 602–606
22. Land, H., Parada, L.F., and Weinberg, R.A. (1983) Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. *Nature* **304**, 596–602
23. Michalovitz, D., Fischer-Fantuzzi, L., Vesco, C., Pipas, J.M., and Oren, M. (1987) Activated Ha-ras can cooperate with defective simian virus 40 in the transformation of nonestablished rat embryo fibroblasts. *J. Virol.* **61**, 2648–2654
24. Ruley, H.E. (1990) Transforming collaborations between ras and nuclear oncogenes. *Cancer Cells* **2**, 258–268
25. DiPaolo, J.A. (1983) Relative difficulties in transforming human and animal cells in vitro. *J. Natl. Cancer Inst.* **70**, 3–8
26. Hahn, W.C., Counter, C.M., Lundberg, A.S., Beijersbergen, R.L., Brooks, M.W., and Weinberg, RA (1999) Creation of human tumour cells with defined genetic elements. *Nature* **400**, 464–468
27. Akagi, T., Sasai, K., and Hanafusa, H. (2003) Refractory nature of normal human diploid fibroblasts with respect to oncogene-mediated transformation. *Proc. Natl. Acad. Sci. USA* **100**, 13567–13572
28. Sukezane, T., Oneyama, C., Kakumoto, K., Shibutani, K., Hanafusa, H., and Akagi, T. (2005) Human diploid fibroblasts are resistant to MEK/ERK-mediated disruption of the actin cytoskeleton and invasiveness stimulated by Ras. *Oncogene* **24**, 5648–5655
29. Akagi, T. (2004) Oncogenic transformation of human cells: shortcomings of rodent model systems. *Trends Mol. Med.* **10**, 542–548
30. Kakumoto, K., Sasai, K., Sukezane, T., Oneyama, C., Ishimaru, S., Shibutani, K., Mizushima, H., Mekada, E., Hanafusa, H., and Akagi, T. (2006) FRA1 is a determinant for the difference in RAS-induced transformation between human and rat fibroblasts. *Proc. Natl. Acad. Sci. USA* **103**, 5490–5495
31. Sasai, K., Kakumoto, K., Hanafusa, H., and Akagi, T. (2007) The Ras-MAPK pathway downregulates Caveolin-1 in rodent fibroblast but not in human fibroblasts: implications in the resistance to oncogene-mediated transformation. *Oncogene* **26**, 449–455
32. Goff, S.P., Gilboa, E., Witte, O.N., and Baltimore, D. (1980) Structure of the Abelson murine leukemia virus genome and the homologous cellular gene: studies with cloned viral DNA. *Cell* **22**, 777–785
33. Wang, J.Y., Ledley, F., Goff, S., Lee, R., Groner, Y., and Baltimore, D. (1984) The mouse c-abl locus: molecular cloning and characterization. *Cell* **36**, 349–356
34. Rosenberg, N. (1994) Abl-mediated transformation, immunoglobulin gene rearrangements and arrest of B lymphocyte differentiation. *Semin. Cancer Biol.* **5**, 95–102
35. Radfar, A., Unnikrishnan, I., Lee, H.W., DePinho, R.A., and Rosenberg, N. (1998) p19(Arf) induces p53-dependent apoptosis during abelson virus-mediated pre-B cell transformation. *Proc. Natl. Acad. Sci. USA* **95**, 13194–13199
36. Unnikrishnan, I., Radfar, A., Jenab-Wolcott, J., and Rosenberg, N. (1999) p53 mediates apoptotic crisis in primary Abelson virus-transformed pre-B cells. *Mol. Cell Biol.* **19**, 4825–4831
37. Cong, F., Zou, X., Hinrichs, K., Calame, K., and Goff, S.P. (1999a) Inhibition of v-Abl transformation by p53 and p19ARF. *Oncogene* **18**, 7731–7739
38. Muljo, S.A. and Schlissel, M.S. (2003) A small molecule Abl kinase inhibitor induces differentiation of Abelson virus-transformed pre-B cell lines. *Nat. Immunol.* **4**, 31–37
39. Renshaw, M.W., Kipreos, E.T., Albrecht, M.R., and Wang, J.Y. (1992) Oncogenic v-Abl tyrosine kinase can inhibit or stimulate growth, depending on the cell context. *EMBO J.* **11**, 3941–3951
40. Sawyers, C.L., McLaughlin, J., Goga, A., Havlik, M., and Witte, O. (1994) The nuclear tyrosine kinase c-Abl negatively regulates cell growth. *Cell* **77**, 121–131
41. Kamijo, T., Zindy, F., Roussel, M.F., Quelle, D.E., Downing, J.R., Ashmun, R.A., Grosveld, G., and Sherr, C.J. (1997) Tumor suppression at the mouse INK4a locus mediated by the alternative reading frame product p19ARF. *Cell* **91**, 649–659
42. Hantschel, O., Nagar, B., Guettler, S., Kretzschmar, J., Dorey, K., Kuriyan, J., and Superti-Furga, G. (2003) A myristoyl/phosphotyrosine switch regulates c-Abl. *Cell* **112**, 845–857
43. Nagar, B., Hantschel, O., Young, M.A., Scheffzek, K., Veach, D., Bornmann, W., Clarkson, B., Superti-Furga, G., and Kuriyan, J. (2003) Structural basis for the autoinhibition of c-Abl tyrosine kinase. *Cell* **112**, 859–871
44. Hantschel, O. and Superti-Furga, G. (2004) Regulation of the c-Abl and Bcr-Abl tyrosine kinases. *Nat. Rev. Mol. Cell Biol.* **5**, 33–44
45. Shi, Y., Alin, K., and Goff, S.P. (1995) Abl-interactor-1, a novel SH3 protein binding to the carboxy-terminal portion of the Abl protein, suppresses v-abl transforming activity. *Genes Dev.* **9**, 2583–2597
46. Ikeguchi, A., Yang, H.Y., Gao, G., and Goff, S.P. (2001) Inhibition of v-Abl transformation in 3T3 cells overexpressing different forms of the Abelson interactor protein Abi-1. *Oncogene* **20**, 4926–4934
47. Tani, K., Sato, S., Sukezane, T., Kojima, H., Hirose, H., Hanafusa, H., and Shishido, T. (2003) Abl interactor 1 promotes tyrosine 296 phosphorylation of mammalian enabled (Mena) by c-Abl kinase. *J. Biol. Chem.* **278**, 21685–21692
48. Maruoka, M., Suzuki, J., Kawata, S., Yoshida, K., Hirao, N., Sato, S., Goff, S.P., Takeya, T., Tani, K., and Shishido, T. (2005) Identification of B cell adaptor for PI3-kinase (BCAP) as an Abl interactor 1-regulated substrate of Abl kinases. *FEBS Lett.* **579**, 2986–2990
49. Shishido, T. and Suzuki, J. (2005) Regulation of Abl kinases by adaptor proteins. *Gene Ther. Mol. Biol.* **9**, 339–342
50. Lin, T.Y., Huang, C.H., Chou, W.G., and Juang, J.L. (2004) Abi enhances Abl-mediated CDC2 phosphorylation and inactivation. *J. Biomed. Sci.* **11**, 902–910
51. Leng, Y., Zhang, J., Badour, K., Arpaia, E., Freeman, S., Cheung, P., Siu, M., and Siminovitch, K. (2005) Abelson-interactor-1 promotes WAVE2 membrane translocation and Abelson-mediated tyrosine phosphorylation required for

WAVE2 activation. *Proc. Natl. Acad. Sci. USA* **102**, 1098–1103
52. Stuart, J.R., Gonzalez, F.H., Kawai, H., and Yuan, Z.M. (2006) c-Abl interacts with the WAVE2 signaling complex to induce membrane ruffling and cell spreading. *J. Biol. Chem.* **281**, 31290–31297
53. Andoniou, C.E., Thien, C.B., and Langdon, W.Y. (1994) Tumour induction by activated abl involves tyrosine phosphorylation of the product of the cbl oncogene. *EMBO J.* **13**, 4515–4523
54. Shishido, T., Akagi, T., Ouchi, T., Georgescu, M.M., Langdon, W.Y., and Hanafusa, H. (2000) The kinase-deficient Src acts as a suppressor of the Abl kinase for Cbl phosphorylation. *Proc. Natl. Acad. Sci. USA* **97**, 6439–6444
55. Feshchenko, E.A., Shore, S.K., and Tsygankov, A.Y. (1999) Tyrosine phosphorylation of C-Cbl facilitates adhesion and spreading while suppressing anchorage-independent growth of V-Abl-transformed NIH3T3 fibroblasts. *Oncogene* **18**, 3703–3015
56. Cong, F., Yuan, B., and Goff, S.P. (1999b) Characterization of a novel member of the DOK family that binds and modulates Abl signaling. *Mol. Cell Biol.* **19**, 8314–8325
57. Feller, S.M., Knudsen, B., and Hanafusa, H. (1994) c-Abl kinase regulates the protein binding activity of c-Crk. *EMBO J.* **13**, 2341–2351
58. Shishido, T., Akagi, T., Chalmers, A., Maeda, M., Terada, T., Georgescu, M.M., and Hanafusa, H. (2001) Crk family adaptor proteins trans-activate c-Abl kinase. *Genes Cells* **6**, 431–440
59. Iwahara, T., Akagi, T., Fujitsuka, Y., and Hanafusa, H. (2004) CrkII regulates focal adhesion kinase activation by making a complex with Crk-associated substrate, p130Cas. *Proc. Natl. Acad. Sci. USA* **101**, 17693–17698
60. Kain, K.H., Gooch, S., and Klemke, R.L. (2003) Cytoplasmic c-Abl provides a molecular 'Rheostat' controlling carcinoma cell survival and invasion. *Oncogene* **22**, 6071–6080
61. Noren, N.K., Foos, G., Hauser, C.A., and Pasquale, E.B. (2006) The EphB4 receptor suppresses breast cancer cell tumorigenicity through an Abl-Crk pathway. *Nat. Cell Biol.* **8**, 815–825
62. Suzuki, J., Sukezane, T., Akagi, T., Georgescu, M.M., Ohtani, M., Inoue, H., Jat, P.S., Goff, S.P., Hanafusa, H., and Shishido, T. (2004) Loss of c-abl facilitates anchorage-independent growth of p53- and RB- deficient primary mouse embryonic fibroblasts. *Oncogene* **23**, 8527–8534
63. Whang, Y.E., Tran, C., Henderson, C., Syljuasen, R.G., Rozengurt, N., McBride, W.H., and Sawyers, C.L. (2000) c-Abl is required for development and optimal cell proliferation in the context of p53 deficiency. *Proc. Natl. Acad. Sci. USA* **97**, 5486–5491
64. Engelman, J.A., Zhang, X.L., Razani, B., Pestell, R.G., and Lisanti, M.P. (1999) p42/44 MAP kinase-dependent and -independent signaling pathways regulate caveolin-1 gene expression. Activation of Ras-MAP kinase and protein kinase a signaling cascades transcriptionally down-regulates caveolin-1 promoter activity. *J. Biol. Chem.* **274**, 32333–32341
65. Engelman, J.A., Wykoff, C.C., Yasuhara, S., Song, K.S., Okamoto, T., and Lisanti, M.P. (1997) Recombinant expression of caveolin-1 in oncogenically transformed cells abrogates anchorage-independent growth. *J. Biol. Chem.* **272**, 16374–16381
66. Lee, H., Volonte, D., Galbiati, F., Iyengar, P., Lublin, D.M., Bregman, D.B., Wilson, M.T., Campos-Gonzalez, R., Bouzahzah, B., Pestell, R.G., Scherer, P.E., and Lisanti, M.P. (2000) Constitutive and growth factor-regulated phosphorylation of caveolin-1 occurs at the same site (Tyr-14) in vivo: identification of a c-Src/Cav-1/Grb7 signaling cassette. *Mol. Endocrinol.* **14**, 1750–1775
67. Corley Mastick, C., Sanguinetti, A.R., Knesek, J.H., Mastick, G.S., and Newcomb, L.F. (2001) Caveolin-1 and a 29-kDa caveolin-associated protein are phosphorylated on tyrosine in cells expressing a temperature-sensitive v-Abl kinase. *Exp. Cell Res.* **266**, 142–154
68. Sanguinetti, A.R. and Mastick, C.C. (2003) c-Abl is required for oxidative stress-induced phosphorylation of caveolin-1 on tyrosine 14. *Cell Signal.* **15**, 289–298
69. del Pozo, M.A., Balasubramanian, N., Alderson, N.B., Kiosses, W.B., Grande-Garcia, A., Anderson, R.G., and Schwartz, M.A. (2005) Phospho-caveolin-1 mediates integrin-regulated membrane domain internalization. *Nat. Cell Biol.* **7**, 901–908
70. Srinivasan, D. and Plattner, R. (2006) Activation of Abl tyrosine kinases promotes invasion of aggressive breast cancer cells. *Cancer Res.* **66**, 5648–5655
71. Nowell, P.C. and Hungerford, D.A. (1960) Chromosome studies on normal and leukemic human leukocytes. *J. Natl. Cancer Inst.* **25**, 85–109
72. Rowley, J.D. (1973) Letter: A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. *Nature* **243**, 290–293
73. Chan, L.C., Karhi, K.K., Rayter, S.I., Heisterkamp, N., Eridani, S., Powles, R., Lawler, S.D., Groffen, J., Foulkes, J.G., Greaves, M.F., and Wiedemann, L.M. (1987) A novel abl protein expressed in Philadelphia chromosome positive acute lymphoblastic leukaemia. *Nature* **325**, 635–637
74. Kurzrock, R., Shtalrid, M., Romero, P., Kloetzer, W.S., Talpas, M., Trujillo, J.M., Blick, M., Beran, M., and Gutterman, J.U. (1987) A novel c-abl protein product in Philadelphia-positive acute lymphoblastic leukaemia. *Nature* **325**, 631–635
75. Walker, L.C., Ganesan, T.S., Dhut, S., Gibbons, B., Lister, T.A., Rothbard, J., and Young, B.D. (1987) Novel chimaeric protein expressed in Philadelphia positive acute lymphoblastic leukaemia. *Nature* **329**, 851–853
76. Clark, S.S., McLaughlin, J., Crist, W.M., Champlin, R., and Witte, O.N. (1987) Unique forms of the abl tyrosine kinase distinguish Ph1-positive CML from Ph1-positive ALL. *Science* **235**, 85–88
77. Calabretta, B. and Perrotti, D. (2004) The biology of CML blast crisis. *Blood* **103**, 4010–4022
78. Asimakopoulos, F.A., Shteper, P.J., Krichevsky, S., Fibach, E., Polliack, A., Rachmilewitz, E., Ben-Neriah, Y., and Ben-Yehuda, D. (1999) ABL1 methylation is a distinct molecular event associated with clonal evolution of chronic myeloid leukemia. *Blood* **94**, 2452–2460
79. Daley, G.Q., McLaughlin, J., Witte, O.N., and Baltimore, D. (1987) The CML-specific P210 bcr/abl protein, unlike v-abl, does not transform NIH/3T3 fibroblasts. *Science* **237**, 532–535
80. Lugo, T.G. and Witte, O.N. (1989) The BCR-ABL oncogene transforms Rat-1 cells and cooperates with v-myc. *Mol. Cell Biol.* **9**, 1263–1270