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Short Communication

The oncogenic activity of the Src family kinase Hck requires the cooperative action of the plasma membrane- and lysosome-associated isoforms

Renaud Poincloux[a,b], *Talal Al Saati*[c], *Isabelle Maridonneau-Parini*[a,b,d,*], *Véronique Le Cabec*[a,b,d,*]

[a]*CNRS, IPBS (Institut de Pharmacologie et de Biologie Structurale), 205 Route de Narbonne, F-31077 Toulouse, France*
[b]*Université de Toulouse, UPS, IPBS, F-31077 Toulouse, France*
[c]*Plateau Technique d'histopathologie expérimentale, Inserm-IFR30/Génopole Toulouse-Midi Pyrénées, Toulouse, France*

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ABSTRACT

Hck is a phagocyte specific proto-oncogene of the Src family expressed as two isoforms, p59Hck and p61Hck. It plays a critical role in Bcr/Abl-chronic myeloid leukaemia and is able to transform fibroblasts *in vitro*. However, the tumourigenic activity of Hck and the respective oncogenic functions of Hck isoforms have not been examined. Tet-Off fibroblasts expressing constitutively active mutants of p59Hck and p61Hck together or individually were used. In contrast to cells expressing p59Hck^{ca} or p61Hck^{ca} alone, cells expressing both isoforms were transformed *in vitro* and induced tumour formation in 90% of nude mice within 2 weeks. This is the first demonstration of (i) the tumourigenic activity of Hck in mice, (ii) the cooperative action of the two Hck isoforms *in vitro* and *in vivo*. To our knowledge, this is the first example of a transforming activity 'split' in two requisite isoforms.

1. Introduction

Src family protein-tyrosine kinases (SFKs) are known to mediate mitogenesis, differentiation, survival, migration, and adhesion.[1] In contrast to the ubiquitous expression pattern of Src, Yes, and Fyn, the expression of Lck, Blk, Lyn, Fgr and Hck is restricted to hematopoietic cells.[2,3]

Like other Src-family kinases, the proto-oncogene Hck harbours an *in vitro* transforming activity in murine fibroblasts[4] upon constitutive activation either by mutation of the negative regulatory tyrosine residue in its carboxy-terminal region to phenylalanine,[1] or by the HIV protein Nef that is a well known ligand of its SH3 domain.[5] To date, spontaneous mutation of this proto-oncogene has not been described in human cancers. Constitutive activation of Hck by direct interaction with the oncogene Bcr/Abl is required for the establishment of leucocyte transformation during chronic myeloid leukaemia (CML).[6–8] Actually, inhibitors of Src-PTKs block the

* *Corresponding authors:* Isabelle Maridonneau-Parini, Université Paul Sabatier, Institut de Pharmacologie et de Biologie structurale, CNRS UMR 5089, 205 route de Narbonne, 31077 Toulouse, France. Tel.: +33 561 17 54 58; fax: +33 561 17 59 94 (V. Le Cabec); tel.: +33 561 17 54 54 (I. Maridonneau-Parini).
E-mail addresses: Isabelle.Maridonneau-Parini@ipbs.fr (I. Maridonneau-Parini), Veronique.Le-Cabec@ipbs.fr (V. Le Cabec).
[d] These authors are co-last authors and co-corresponding authors.
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transformation process in Bcr/Abl-expressing cells and, in myeloid cells, transformation by Bcr/Abl is suppressed in Hck-defective cells.[6,9] The recent demonstration of a Bcr/Abl-independent, SFKs-dependent form of resistance to imatinib, a specific Bcr/Abl inhibitor used to treat CML patients,[10] has further supported the central role of SFKs in this leukaemia, which are therefore considered as rational targets for anti-CML therapy either alone or in combination with Bcr/Abl inhibitors.[11]

Hck is specifically expressed in myeloid cells as two isoforms of molecular weights 59 kDa (p59Hck) and 61 kDa (p61Hck) generated by alternative initiations of translation of a single mRNA.[12] The isoforms only differ by 21 residues at the N-terminal end, and by their differential addressing, p59Hck being mainly associated to the plasma membrane and p61Hck to the lysosome membrane.[13,14] Expression of the constitutively activated variant of p61Hck (p61Hck^{ca}) induces the *de novo* formation of podosome rosettes in a lysosome-dependent manner,[15] whereas p59Hck^{ca} triggers the formation of plasma membrane protrusions.[16] Thus, although very similar, p59Hck and p61Hck are differentially located and trigger distinct phenotypes when activated.

Despite the growing pharmacological interest of Hck, its oncogenic activity has been very poorly documented since its discovery 20 years ago. The aim of our work is to investigate the tumourigenic ability of Hck, which has not been investigated yet, and to examine the respective contribution of p59Hck and p61Hck in the transformation process *in vitro* and *in vivo*. Therefore, we used murine MEF3T3 Tet-Off fibroblasts that stably and conditionally expressed constitutively active isoforms of the kinase. This cellular model has the advantage of avoiding long-lasting- as well as over-expression of the oncogenic Hck mutant. We herein report that the combined expression of p59Hck and p61Hck is necessary for the full exercise of its transforming and tumourigenic activities.

2. Materials and methods

2.1. Antibodies

Antibodies directed against Hck and GFP (used at final dilution 1:200 and 1:100, respectively) were purchased from Santa Cruz Biotechnology (Tebu-bio, Le Perray-en-Yvelines, France) and Abcam (ab6556, Abcam, Paris, France). Texas-Red Phalloidin was from Molecular Probes (Leiden, The Netherlands).

2.2. Constructs, cells and cultures

Constitutively active (HckY501F) mutants of human p59Hck^{ca}, p61Hck^{ca} and p59/61Hck^{ca} have been previously described [15–17]. For doxycyline regulatable oncogene expression, we used mouse embryonic fibroblast 3T3 (MEF-3T3) Tet-Off cell line (Clontech) that were grown, transfected and cloned as described.[15,17] Hck expression was optimal in doxycycline-free medium after 7 days. Several clones were selected: MEF-p59, MEF-p61 and MEF-p59/61 clones A and B, expressing p59Hck^{ca}-GFP, p61Hck^{ca}-GFP and both p59 and p61Hck^{ca}-GFP respectively. Hck negative-MEF-3T3 Tet-Off (Parental-MEF) was used as a negative control in transformation experiments *in vitro*.

2.3. SDS-PAGE and immunoblot analysis

Equal amounts of total cell lysates in Laemmli buffer were separated by SDS-PAGE, transferred to nitrocellulose membranes that were probed with polyclonal rabbit anti-Hck antibodies (0.4 μg/ml) and revealed by an enhanced chemiluminescence system (Immobilon^{TM} Western, Millipore corporation, Billerica, U.S.A.) as described.[17]

2.4. Direct fluorescence and F-actin staining

Cells were plated on glass coverslips for 24 h, fixed and processed as described for F-actin staining using Texas-Red phalloidin.[18] F-actin labelling and direct Hck-GFP fluorescence were detected with a Leica DM-RB fluorescence microscope or a Leica TCS-SP2 confocal scanning microscope.

2.5. In vitro transformation assays

Proliferation curves. Growth curves were generated over 2 weeks for Hck-positive and Hck-negative MEF3T3 stable clones as previously described [19]. Proliferation rate is expressed as the doubling time in days.

Adhesion capacity. Adhesion defect of transformed cells was assessed by direct light microscopy observations of cell detachment once they reached confluency after plating on glass coverslips.

Dependence on serum growth factors. Cell proliferation in culture medium containing 10% FCS was compared to proliferation in 0.5% FCS and measured by counting the cells every day.

Focus forming activity. 5×10^4 cells were seeded on fibrinogen-coated glass coverslips in 24 well-plates. Foci were observed at 10 and 20 days of culture.[19]

Anchorage-independent proliferation. Colony formation in soft agar was followed for 18 days as described.[4] Briefly, 10^4 cells were seeded in DMEM containing 10% FCS and 0.35% w/v low-melting temperature (LMP) agarose. This suspension was sandwiched between layers of DMEM containing 0.7% w/v LMP agarose. Colonies were photographed by phase contrast and fluorescence microscopy and counted.

2.6. In vivo tumourigenicity assay

10^6 cells were inoculated subcutaneously into the flank of 6-week-old male athymic SWISS nude mice (Charles River, France). Tumour development was assessed every week until the tumour was removed for histopathological analysis or until 4 months post-injection for negative mice. Tumours were measured every 5 days from day 21 to day 37. To study the development of metastasis in nude mice, primary tumours were induced as described above and after 2 to 4 weeks (depending on the size of the tumour), primary tumours were removed and mice follow-up for metastatic development was undertaken.

2.7. Histopathological analysis and immunohistochemistry

Subcutaneous tumours were fixed in 10% v/v neutral buffered formalin and embedded in paraffin. Sections were stained

with haematoxylin and eosin for histomorphological analyses. Immunohistochemical staining was performed on paraffin-embedded tissue sections, using rabbit polyclonal anti-GFP antibodies. Immunostaining of paraffin sections was preceded by antigen retrieval technique by microwave heating twice in 10 mM citrate buffer, pH6. After 30 min incubation with the antibodies at room temperature, sections were incubated with biotin-conjugated polyclonal anti-rabbit immunoglobulin antibodies followed by streptavidin-biotin-peroxidase complex (Vector Laboratories, Burlingame, CA) and counter-staining with haematoxylin. Negative controls were incubated in buffered solution without primary antibody.

3. Results

3.1. *The expression of both p59Hck and p61Hck is required for cell transformation in vitro*

To assess the respective roles of p59Hck and/or p61Hck in the transforming activity of Hck, we developed murine fibroblast MEF3T3 Tet-Off cells stably expressing each Hck isoform separately or jointly under their constitutively active (Hck^{ca}) forms and in fusion with GFP. The advantage of using the Tet-Off regulation is to control the expression of oncogenes to avoid cell line degeneration. Several MEF3T3 Tet-Off clones that stably expressed at a quantitatively comparable level either $p59Hck^{ca}$ or $p61Hck^{ca}$ alone or both Hck^{ca} isoforms were selected (Fig. 1A). We have previously reported that the expression level in MEF-p59/61Hck is comparable to endogenous Hck expressed in primary human neutrophils.[17] As shown in Fig. 1B, these cell lines exhibited characteristic F-actin reorganisation due to Hck expression[15,16]: $p59Hck^{ca}$ expression triggered plasma membrane protrusions, $p61Hck^{ca}$ expression triggered rosette of podosome formation, and concomitant expression of $p59Hck^{ca}$ and $p61Hck^{ca}$ induced the formation of both cellular structures. As expected, clones expressing $p59Hck^{ca}$ and $p61Hck^{ca}$ were transformed (Table 1) as revealed by: (i) adhesion defects: they weakly attach to glass coverslips once they reach confluency, (ii) the ability to grow in the absence of serum and (iii) in the absence of anchorage (Fig. 2A), (iv) a loss of contact inhibition

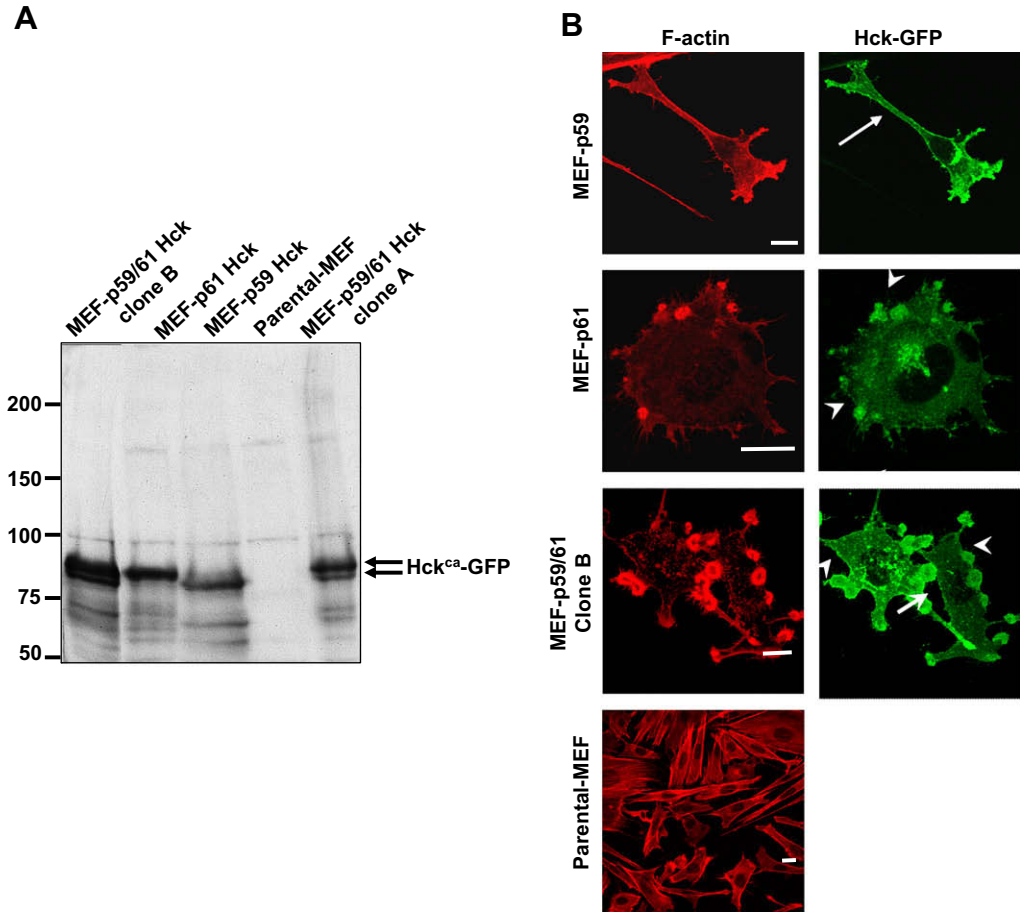

Fig. 1 – Characterisation of MEF3T3 Tet-Off clones expressing $p59Hck^{ca}$ and /or $p61Hck^{ca}$. (A) Hck is expressed at comparable levels in MEF3T3 clones: total cell lysates prepared from parental cells (Parental-MEF), cells expressing $p59Hck^{ca}$ (MEF-p59), $p61Hck^{ca}$ (MEF-p61) or both $p59Hck^{ca}$ and $p61Hck^{ca}$ (MEF-p59/61 clone A and clone B) were subjected to Western blot analysis. Equal amounts of proteins were loaded in each well. Molecular weights are indicated in kDa. (B) MEF3T3 Tet-Off clones exhibit the characteristic Hck phenotype. Hck-GFP and F-actin stained by texas-red phalloidin were visualised using a Leica TCS-SP2 confocal scanning microscope. Scale bars: 10 μm.

Table 1 – Summary of the transforming and tumourigenic activities of Hck-expressing cells.

	Parental cells	p59Hck^{ca}	p61Hck^{ca}	p59/61Hck^{ca}
Doubling time (days)	1.63+0.13	1.51+0.58	1.73+0.60	1.11+0.16
Adhesion defect	–	–	–	+
Loss of contact inhibition	–	–	–	+
Anchorage independent growth	–	–	–	+
Serum independent growth	–	–	–	+
Tumour incidence	0/10 mice within 4 months	0/10 mice within 4 months	0/9 mice within 4 months	18/20 mice 10 to 65 days

(Fig. 2B), and (v) a shortened doubling time. Importantly, when Hck expression was induced during 7 to 21 days and secondly repressed before performing the soft-agar assay, cells lost their capacity to form colonies indicating that cell transformation was reversible and only dependent on Hck^{ca} expression. In contrast, cells that expressed either p59Hck^{ca} or p61Hck^{ca} alone did not exhibit transformed features (Table 1 and Fig. 2B), showing that the cooperative action of both Hck isoforms is necessary to express its oncogenic activity.

3.2. *Hck is a powerful oncogene in vivo requiring the expression of both p59Hck and p61Hck*

Since the tumourigenic ability of Hck has never been investigated, we tested *in vivo* the tumourigenic action of each isoform alone or in combination. Hck-transformed MEF3T3 cells (clone B) were injected into athymic nude mice. Exponentially growing tumours appeared in 90% of injected mice (Table 1) two weeks after injection (Fig. 3A). Similar results were obtained when MEF-p59/61 clone A was used (data not shown). To demonstrate that tumours were only derived from Hck expressing MEF3T3 cells, tumours were analysed for recombinant Hck-GFP expression either by direct GFP fluorescence on frozen tumour tissue sections or by immunohistochemistry using anti-GFP antibodies on paraffin-embedded tissue sections. Both techniques confirmed that all tumour cells were Hck-GFP positives (Fig. 3B). Haematoxylin-eosin staining of tumour sections revealed tumour cells infiltration into the skin and the subcutaneous muscle layer (Fig. 3C), which illustrates the local invasion capacity of Hck-transformed cells, but no

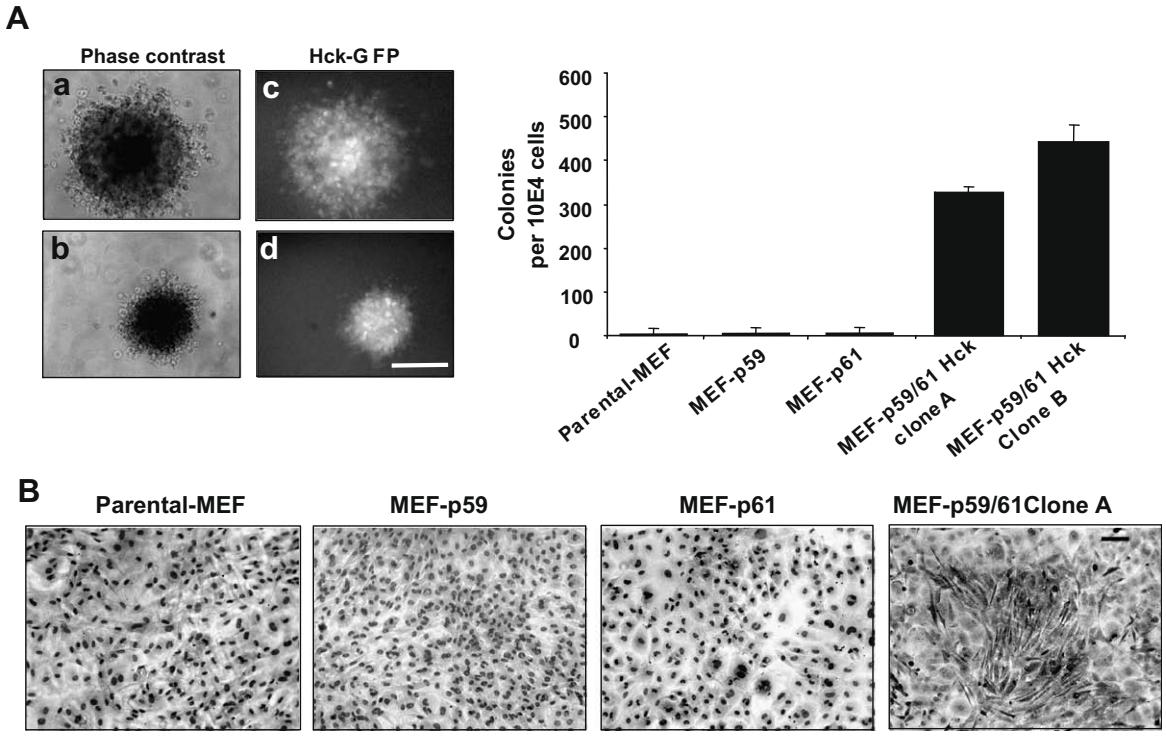

Fig. 2 – MEF3T3 Tet-Off cells expressing both Hck isoforms are transformed *in vitro*. (A) Anchorage-independent growth of MEF3T3 Tet-Off cells. Colonies formed by MEF-p59/61 clone A in soft agar were photographed under transmitted light (a,b) or fluorescence microscopy (c,d) for Hck-GFP expression. Histograms show the results representative of three independent experiments quantifying colony formation for Parental-MEF, MEF-p59, MEF-p61 or MEF-p59/61 clone A and B. (B) Loss of contact inhibition was examined by plating Parental-MEF, MEF-p59, MEF-p61 or MEF-p59/61 clone A at confluence at day zero and observing foci formation after 10 days. MEF-p59/61 cells form foci in contrast to Parental-MEF, MEF-p59 or MEF-p61 cells. Pictures show cells stained with May-Grünwald Giemsa. Scale bars: 500 μm in A and 50 μm in B.

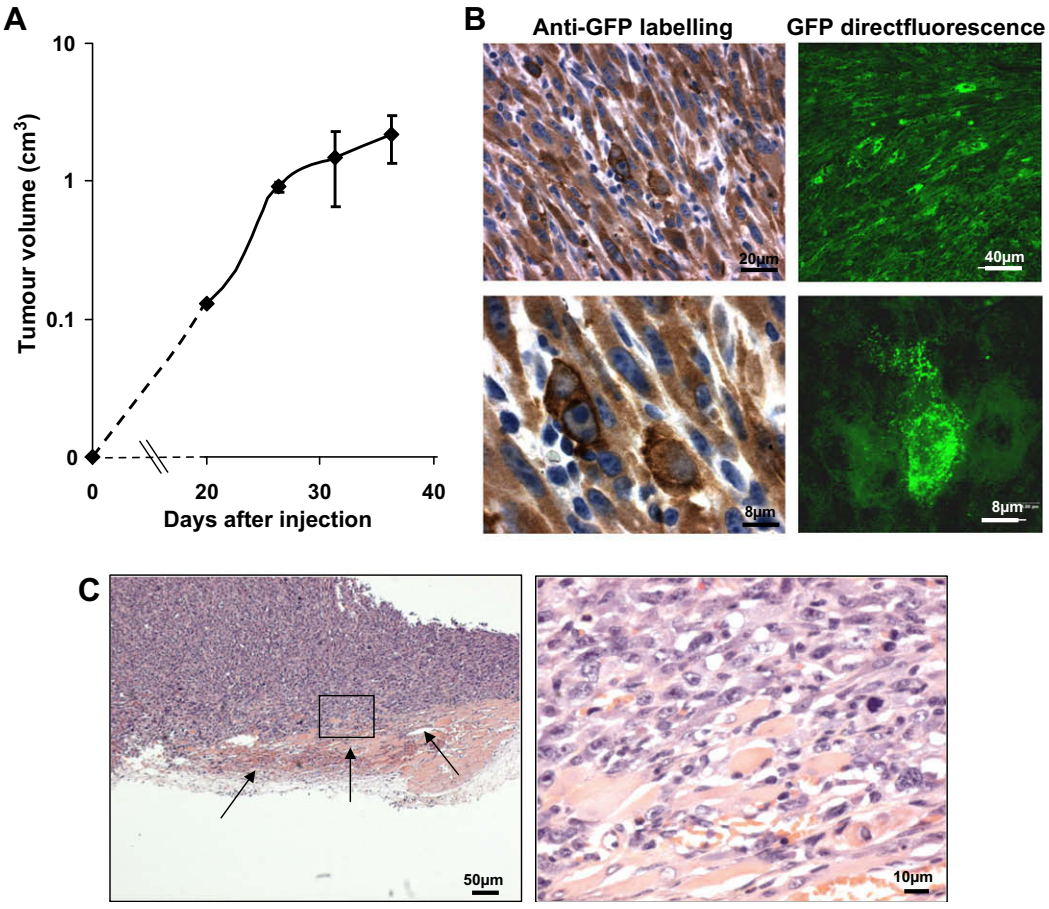

Fig. 3 – Tumourigenic effect of p59/61Hck^{ca} expressing cells. (A) 2×10^6 MEF-p59/61 clone B cells were injected subcutaneously into the flank of male nude mice. Tumour volumes were measured at indicated time points from the 2nd to the 4th week after the injection on several mice (values represent the mean ± SE from a total of 10). (B) Tumours were removed and analysed for Hck-GFP expression by immunohistochemistry (left panels) or direct GFP fluorescence on frozen tumour tissue sections visualised using confocal microscopy (right panels). (C) Haematoxylin-eosin staining of formalin fixed paraffin-embedded tumour tissue sections shows that tumour cells displayed diffuse infiltration of the skin and subcutaneous muscle tissues. Right picture is a zoom of the square in the left picture. Arrows indicate tumour cell invasion into the adjacent skin muscle layer resulting in destruction of muscle tissue.

clinical sign of metastasis development was observed, even after removal of the primary tumour to lengthen the mice follow-up. In contrast to MEF-p59/p61Hck but like parental cells, p59Hck- or p61Hck-expressing cells were not able to induce tumour formation even 4 months after injection (Table 1), thus confirming the necessity of both Hck isoforms for expression of the oncogenic activity of the kinase.

4. Discussion

This is the first report showing that joint expression of constitutively active Hck isoforms is essential for promoting cell proliferation and tumourigenic activity in mice. This first example of a transforming activity 'split' in two requisite isoforms suggests that the activity of other oncogenes could be the result of the coordinated action of different isoforms.

Few proto-oncogenes are expressed as multiple isoforms, and usually, these isoforms are not co-expressed in the same cells. Ras, the paradigm for proto-oncogenes, is expressed as multiple isoforms in the same cells. Ras isoforms originate either from distinct genes or from alternative splicing and share a high degree of sequence homology.[20] Most studies have focused on the K-Ras isoform because of its strong transforming activity, and little is known on the effects of the other isoforms. Like Hck, Ras isoforms are differentially localised in cells and participate in distinct signalling pathways. However, in contrast to Hck, Ras isoforms trigger cell transformation when individually expressed under their oncogenic state.[20]

Lyn is the other Src family proto-oncogene expressed as two transcription variants in the same tissue and in different subcellular compartments[21] but the role played by each isoform in its transforming activity has not been examined. Src and Fgr, the cellular counterparts of two viral oncogenes, are expressed as unique proteins but with dual subcellular localisations, at the plasma membrane and on intracellular vesicles.[22–24] Whether the transforming activity of these SFKs requires their association with both membrane types,

potentially giving them access to different substrates, is an interesting hypothesis.

Hck isoforms are differentially addressed to distinct intracellular compartments.[14] Although both Hck isoforms share 95% identity and have the ability to reorganise the actin cytoskeleton, distinct phenotypes are obtained probably reflecting distinct access to available substrates. p59Hck is associated to the plasma membrane where it triggers protrusions and thus is likely the isoform involved in the downstream signalling of several receptors. p61Hck is associated with the membrane of lysosomes where it triggers the formation of actin comets[25] and the formation of podosome rosettes,[15] structures involved in cell adhesion and extracellular matrix degradation. Whether signalling from plasma membrane and from vesicular compartments is relevant for cell transformation needs further investigation. The signalling pathways leading to cell transformation induced by some SFKs have been broadly unravelled.[21] Oncogenic forms of Src transmit signals that regulate proliferation and cell survival.[21] Cell migration is also altered, in particular the actin cytoskeleton and the adhesion networks are disturbed.[21] How Hck mediates key biological events associated to transformation is not yet understood. Like Src, it has been shown to regulate the actin cytoskeleton.[25,26] Overexpression of wild type Hck isoforms does not trigger any obvious change in the actin organisation indicating that constitutive activation of the kinase is required (data not shown). Hck^{ca} is able to phosphorylate several actin-regulating proteins such as WASp,[27] WIP[28] and Vav.[29] In Bcr/Abl positive leukaemia cells, Hck regulates the phosphorylation status of the transcription factor STAT5.[6] In constitutively active Stat5 mutant-expressing leukaemic cells, tyrosine phosphorylated STAT5 is retained in the cytoplasm, a critical step in the cell transformation process, and we have shown that tyrosine phosphorylated STAT5 locates at actin-rich podosomes in Hck-activated cells.[17,30] Whether the cytoskeleton modifications induced by Hck isoforms can be related to cell motility and invasion is not known. Despite the capacity of Hck-transformed cells to invade tissues surrounding the tumours (Fig. 3), no clinical sign of metastasis has been observed suggesting that Hck is not able to induce all the phases of metastases development.

It is now clear that Hck oncogenic activity has to be considered in terms of the contributions of individual isoforms rather than of a collective whole. Identification of specific p59Hck and p61Hck effectors is a challenge to better understand the complementary role they play in the transforming activity of Hck.

Conflict of interest statement

None declared.

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REFERENCES

1. Brown MT, Cooper JA. Regulation, substrates and functions of src. *Biochim Biophys Acta* 1996;**1287**(2–3):121–49.
2. Quintrell N, Lebo R, Varmus H, et al. Identification of a human gene (HCK) that encodes a protein-tyrosine kinase and is expressed in hemopoietic cells. *Mol Cell Biol* 1987;**7**(6):2267–75.
3. Ziegler SF, Marth JD, Lewis DB, Perlmutter RM. Novel protein-tyrosine kinase gene (hck) preferentially expressed in cells of hematopoietic origin. *Mol Cell Biol* 1987;**7**(6):2276–85.
4. Ziegler SF, Levin SD, Perlmutter RM. Transformation of NIH 3T3 fibroblasts by an activated form of p59hck. *Mol Cell Biol* 1989;**9**(6):2724–7.
5. Briggs SD, Sharkey M, Stevenson M, Smithgall TE. SH3-mediated Hck tyrosine kinase activation and fibroblast transformation by the Nef protein of HIV-1. *J Biol Chem* 1997;**272**(29):17899–902.
6. Klejman A, Schreiner SJ, Nieborowska-Skorska M, et al. The Src family kinase Hck couples BCR/ABL to STAT5 activation in myeloid leukemia cells. *Embo J* 2002;**21**(21):5766–74.
7. Danhauser-Riedl S, Warmuth M, Druker BJ, Emmerich B, Hallek M. Activation of Src kinases p53/56lyn and p59hck by p210bcr/abl in myeloid cells. *Cancer Res* 1996;**56**(15):3589–96.
8. Stanglmaier M, Warmuth M, Kleinlein I, Reis S, Hallek M. The interaction of the Bcr-Abl tyrosine kinase with the Src kinase Hck is mediated by multiple binding domains. *Leukemia* 2003;**17**(2):283–9.
9. Lionberger JM, Wilson MB, Smithgall TE. Transformation of myeloid leukemia cells to cytokine independence by Bcr-Abl is suppressed by kinase-defective Hck. *J Biol Chem* 2000;**275**(24):18581–5.
10. Donato NJ, Wu JY, Stapley J, et al. BCR-ABL independence and LYN kinase overexpression in chronic myelogenous leukemia cells selected for resistance to STI571. *Blood* 2003;**101**(2):690–8.
11. Lee F, Fandi A, Voi M. Overcoming kinase resistance in chronic myeloid leukemia. *Int J Biochem Cell Biol* 2008;**40**(3):334–43.
12. Lock P, Ralph S, Stanley E, Boulet I, Ramsay R, Dunn AR. Two isoforms of murine hck, generated by utilization of alternative translational initiation codons, exhibit different patterns of subcellular localization. *Mol Cell Biol* 1991;**11**:4563–70.
13. Möhn H, Le Cabec V, Fischer S, Maridonneau-Parini I. The src-family protein-tyrosine kinase p59hck is located on the secretory granules in human neutrophils and translocates towards the phagosome during cell activation. *Biochem J* 1995;**309**(Pt 2):657–65.
14. Carréno S, Gouze ME, Schaak S, Emorine LJ, Maridonneau-Parini I. Lack of palmitoylation redirects p59hck from the plasma membrane to p61hck-positive lysosomes. *J Biol Chem* 2000;**275**(46):36223–9.
15. Cougoule C, Carreno S, Castandet J, et al. Activation of the lysosome-associated p61Hck isoform triggers the biogenesis of podosomes. *Traffic* 2005;**6**(8):682–94.
16. Carreno S, Caron E, Cougoule C, Emorine LJ, Maridonneau-Parini I. P59Hck isoform induces F-actin reorganization to form protrusions of the plasma membrane in a Cdc42- and Rac-dependent manner. *J Biol Chem* 2002;**277**(23):21007–16.
17. Poincloux R, Cougoule C, Daubon T, Maridonneau-Parini I, Le Cabec V. Tyrosine-phosphorylated STAT5 accumulates on podosomes in Hck-transformed fibroblasts and chronic myeloid leukemia cells. *J Cell Physiol* 2007;**213**(1):212–20.

18. Poincloux R, Vincent C, Labrousse A, et al. Re-arrangements of podosome structures are observed when Hck is activated in myeloid cells. *Eur J Cell Biol* 2006;**85**(3–4):327–32.
19. Johnson KC, Kissil JL, Fry JL, Jacks T. Cellular transformation by a FERM domain mutant of the Nf2 tumour suppressor gene. *Oncogene* 2002;**21**(39):5990–7.
20. Omerovic J, Laude AJ, Prior IA. Ras proteins: paradigms for compartmentalised and isoform-specific signalling. *Cell Mol Life Sci* 2007;**64**(19–20):2575–89.
21. Frame MC. Newest findings on the oldest oncogene; how activated src does it. *J Cell Sci* 2004;**117**(Pt 7):989–98.
22. Gutkind JS, Robbins KC. Translocation of the FGR protein-tyrosine kinase as a consequence of neutrophil activation. *Proc Natl Acad Sci USA* 1989;**86**(22):8783–7.
23. Stenberg PE, Pestina TI, Barrie RJ, Jackson CW. The Src family kinases, Fgr, Fyn, Lck, and Lyn, colocalize with coated membranes in platelets. *Blood* 1997;**89**(7):2384–93.
24. Bjorge JD, Jakymiw A, Fujita DJ. Selected glimpses into the activation and function of Src kinase. *Oncogene* 2000;**19**(49):5620–35.
25. Vincent C, Maridonneau-Parini I, Le Clainche C, Gounon P, Labrousse A. Activation of p61Hck triggers WASp- and Arp2/3-dependent actin-comet tail biogenesis and accelerates lysosomes. *J Biol Chem* 2007;**282**(27):19565–74.
26. Guiet R, Poincloux R, Castandet J, et al. Hematopoietic cell kinase (Hck) isoforms and phagocyte duties – from signaling and actin reorganization to migration and phagocytosis. *Eur J Cell Biol* 2008;**87**(8–9):527–42.
27. Cory GO, Garg R, Cramer R, Ridley AJ. Phosphorylation of tyrosine 291 enhances the ability of WASp to stimulate actin polymerization and filopodium formation. Wiskott-Aldrich Syndrome protein. *J Biol Chem* 2002;**277**(47):45115–21.
28. Scott MP, Zappacosta F, Kim EY, Annan RS, Miller WT. Identification of novel SH3 domain ligands for the Src family kinase Hck. Wiskott-Aldrich syndrome protein (WASP), WASP-interacting protein (WIP), and ELMO1. *J Biol Chem* 2002;**277**(31):28238–46.
29. English BK, Orlicek SL, Mei Z, Meals EA. Bacterial LPS and IFN-gamma trigger the tyrosine phosphorylation of vav in macrophages: evidence for involvement of the hck tyrosine kinase. *J Leukoc Biol* 1997;**62**(6):859–64.
30. Harir N, Pecquet C, Kerenyi M, et al. Constitutive activation of Stat5 promotes its cytoplasmic localization and association with PI3-kinase in myeloid leukemias. *Blood* 2007;**109**(4):1678–86.