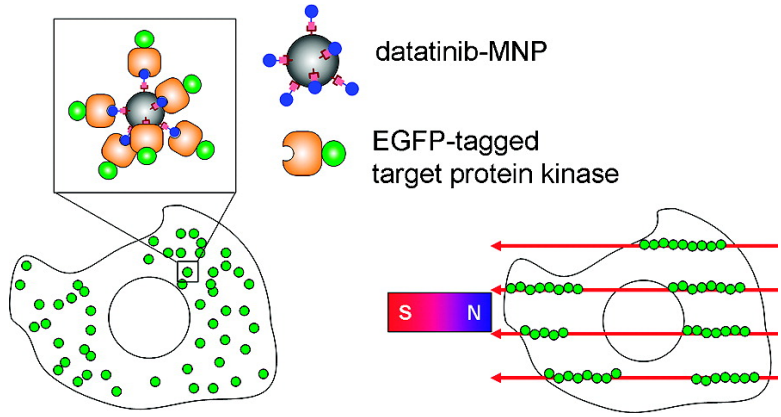
Communication

In Situ Monitoring of Bindings between Dasatinib and Its Target Protein Kinases Using Magnetic Nanoparticles in Live Cells

Dae-Joong Kim, Yong-Weon Yi, and Jin Hwan Kim

J. Am. Chem. Soc., **2008**, 130 (49), 16466-16467 • DOI: 10.1021/ja8063843 • Publication Date (Web): 14 November 2008



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 11/14/2008

In Situ Monitoring of Bindings between Dasatinib and Its Target Protein Kinases Using Magnetic Nanoparticles in Live Cells

Dae-Joong Kim, Yong-Weon Yi, and Jin Hwan Kim*

CGK Co., Ltd., Daejeon Bioventure Town, 461-8, Jeonmin-Dong, Yuseong-Gu, Daejeon, 305-811, Korea

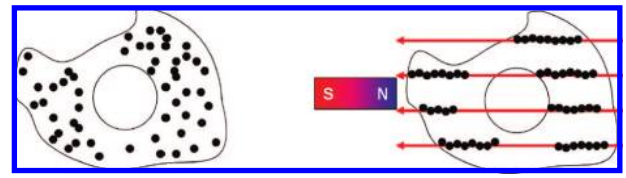
Received August 12, 2008; E-mail: jinhkim1@gmail.com

A thorough understanding of the binding characteristics of a small molecule against its target proteins in live cells is of utmost importance in designing therapeutic agents with improved selectivity and specificity.[1] Cell-based assays using yeast or mammalian three hybrid systems have been used to study the binding characteristics of a small molecule kinase inhibitor and its target protein kinases.[2] Although these three hybrid assays can be performed in a cellular context, the binding characteristics found are related with indirect reporter activities through a cascade of transcriptional and translational events, rather than direct interactions between a small molecule and its target proteins.[2] Here, we have developed a new methodology, called *In Cell Interaction Trap (InCell IT)*, which allows real time monitoring of the direct molecular interactions between a small molecule and its target proteins in live cells using magnetic nanoparticles (MNPs) (Scheme 1). MNPs with a biocompatible coating are widely used for cell labeling in laboratories and MRI contrasting in clinics.[3]

For *InCell IT*, first, MNPs were coated with biocompatible materials attached to streptavidin, followed by mixing with the biotinylated small molecule of interest. Then, these MNPs were transferred by using a protein transduction domain (PTD; Supporting Information) into living cells, and their behaviors within the cells were being monitored under magnetic fields by differential interference contrast (DIC) images with a microscope (Figure 1). After incubation with MNPs, the dark vesicles were observed inside the cells (Figure 1), and iron-specific Prussian blue stain[4] confirmed that these vesicles contained the internalized MNPs (Figure S1). In the absence of magnetic fields, these vesicles containing MNPs showed punctuated localization at the nuclear periphery (Figure 1, left). Applying the magnetic fields in a horizontal direction resulted in the linear alignment of the vesicles to the same direction of the magnetic fields (Figure 1, right and Figure S2), which could be redirected by altering the directions of the magnetic fields (Figure S3). For visualization of interactions between the small molecule on MNPs and its target proteins, the cells expressing the target proteins fused with the EGFP (enhanced green fluorescence protein) tag were used.

The protein kinase family has more than 500 members, and designing selective inhibitors against specific protein kinase isoforms becomes critical in new drug discovery, to improve its efficacy and reduce toxicity.[1] We chose dasatinib (Sprycel, BMS-354825, Bristol-Myers Squibb)[5] as a model compound to evaluate our *InCell IT* for *in situ* monitoring of its binding to target protein kinases, SRC, ABL1, and CSK, in live HeLa cells. Biotinylated dasatinib (Figure S4) showed inhibitory effects against purified human SRC protein kinase *in vitro* on a concentration-dependent manner (Figure S5). The *in vitro* affinity purification studies also demonstrated that the biotinylated dasatinib binds specifically to EGFP-tagged human SRC (SRC-EGFP) or CSK (CSK-EGFP) protein kinases (Figure S6).

Scheme 1. Behavior of Vesicles Containing MNPs in a Live Cell in the Absence or Presence of Magnetic Fields



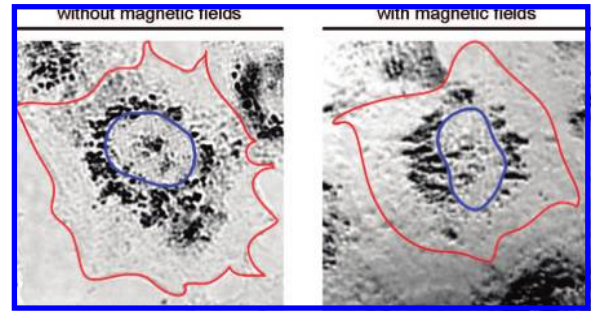


Figure 1. Differential interference contrast (DIC) images showing dark vesicles containing the internalized MNPs and their behaviors in the absence or presence of magnetic fields in the cell. Blue and red lines indicate the boundary of nucleus and cell, respectively.

The streptavidin-attached MNPs were mixed with biotinylated dasatinib (dasatinib-MNPs) and transferred by using PTD into HeLa cells expressing CSK-EGFP. Upon applying magnetic fields, the vesicles containing the internalized dasatinib-MNPs became aligned to the same direction of the magnetic fields however, the fluorescence signals of CSK-EGFP did not colocalize with the dasatinib-MNPs in the cells (Figure 2, left, and Figure S7). These dark vesicular compartments were also detected, when CSK-EGFP expressing cells were transferred with biotin-only coated MNPs (biotin-MNP; Figure S7). On the contrary, no such vesicular compartments were observed in the cells expressing CSK-EGFP without treatment of MNPs (Figure S7). These results suggested that the internalization of MNPs into the cells occurred via endocytosis and the dasatinib-MNPs were further entrapped within membranous vesicles, keeping them from binding with CSK-EGFP in cytoplasm.

Several attempts to release or expose the contents of vesicular compartments to cytoplasm of cells were reported.[6] Among them, we found that incubation of cells with a nordihydroguaiaretic acid (NDGA)[6b] markedly enhanced the cytoplasmic exposure of dasatinib-MNPs entrapped within these vesicles (Figure 2, right). Prior to the addition of NDGA, CSK-EGFP signals were detected separated from the dasatinib-MNP-containing vesicles within the cells (Figure 2, left). Incubation of cells with NDGA, however, enabled CSK-EGFP signals concentrating around dasatinib-MNPs, resulting in linear alignments of CSK-EGFP signals in the same

10.1021/ja8063843 CCC: $40.75

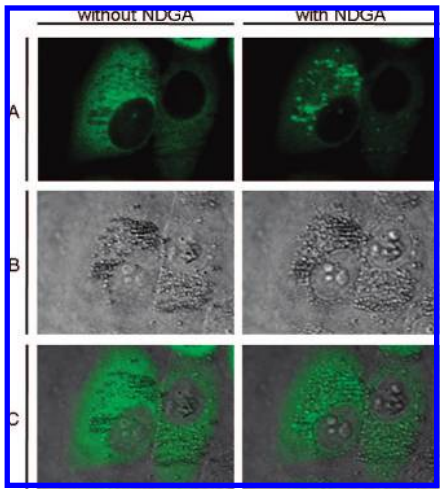


Figure 2. Fluorescence images of cells expressing CSK-EGFP before and after incubation of cells with NDGA under magnetic fields (A), DIC images showing the aligned dasatinib-MNPs in the direction of magnetic fields (B), and the overlapped images of A and B (C).

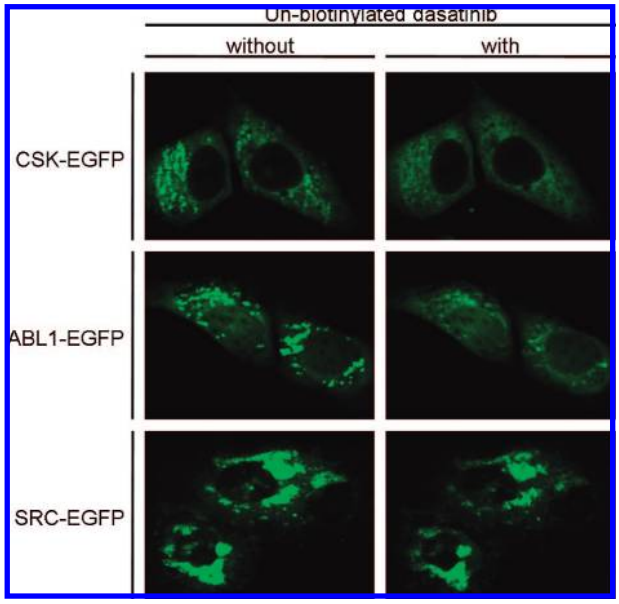
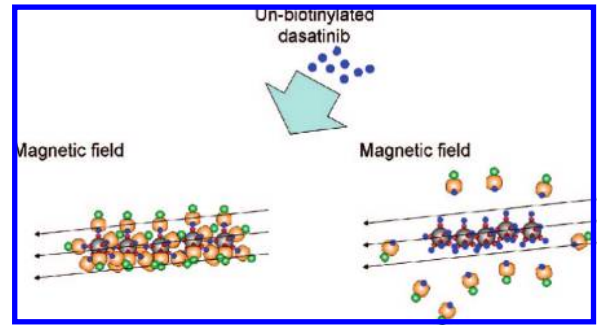


Figure 3. Bindings between dasatinib-MNPs and CSK-, ABL1-, or SRC-EGFPs in the absence or presence of unbiotinylated dasatinib in the same cells.

direction of the magnetic fields in the cells (Figure 2, right). These alignments of CSK-EGFP signals were not observed when the cells were incubated with biotin-MNPs (Figure S7). Binding between dasatinib and human ABL1-EGFP or SRC-EGFP, respectively, was also confirmed in live cells using *InCell IT* (Figure 3, left).

To further demonstrate the specificity of bindings between dasatinib-MNP and its target proteins, competition studies were performed using unbiotinylated dasatinib (Scheme 2). The extent of the binding signals between dasatinib-MNPs and CSK-EGFPs, SRC-EGFPs, or ABL1-EGFPs was decreased substantially by brief coincubation of excess unbiotinylated dasatinib (10 μM)

Scheme 2. Schematic Illustrations of Competition against Dasatinib-MNPs with Unbiotinylated Dasatinib



in the same cells (Figure 3, right). Dose-dependent inhibition of these bindings was achieved when the cells were preincubated with unbiotinylated dasatinib for 1−2 h prior to the addition of NDGA (Figure S8). These bindings in the cells can be preserved by cell fixation with mild fixatives such as formaldehyde, which is useful for visualization of the drug interactions at a particular point in time (Figure S9).

In conclusion, for in situ monitoring of bindings between a small molecule and its target proteins in live cells, we have developed a new technology, *InCell IT*, and examined its applicability using dasatinib, a kinase inhibitor, as a model compound. In *InCell IT*, dasatinib-MNPs were transferred into HeLa cells and their specific bindings to SRC, ABL1, or CSK with EGFP-tag could be monitored under magnetic fields by live cell imaging with a fluorescence microscope. The specificities of these bindings were further confirmed in the same cells by treating unbiotinylated dasatinib itself. *InCell IT* is a new method for detecting the occurrence of small molecule−target protein interactions in live cells.

Acknowledgment. We thank Dr. Yonggil Kwon for critical review and comments on the manuscript. We also thank Dr. Je-Wook Lee for *in vitro* affinity purification studies. This work was supported by CGK Co., Ltd.

Supporting Information Available: Detailed Experimental Procedures, Supporting Figures 1−9, complete ref 2a, and Authors' Note on Previous Work. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Cohen, P. *Nat. Rev. Drug Discovery* **2002**, *1*, 309–315. (b) Hanks, S. K. *Genome Biol.* **2003**, *4*, 111. (c) Knight, Z. A.; Shokat, K. M. *Chem. Biol.* **2005**, *12*, 621–637. (d) Force, T.; Kuida, K.; Namchuk, M.; Parang, K.; Kyriakis, J. M. *Circulation* **2004**, *109*, 1196–1205.
(2) (a) Becker, F.; et al. *Chem. Biol.* **2004**, *11*, 211–223. (b) Caliguiri, M.; Molz, L.; Liu, Q.; Kaplan, F.; Xu, J. P.; Majeti, J. Z.; Ramos-Kelsey, R.; Murthi, K.; Lievens, S.; Tavernier, J.; Kley, N. *Chem. Biol.* **2006**, *13*, 711–722.
(3) Berry, C. C.; Curtis, A. S. G. *J. Phys. D: Appl. Phys.* **2003**, *36*, R198−R206.
(4) Frank, J. A.; Miller, B. R.; Arbab, A. S.; Zywicke, H. A.; Jordan, E. K.; Lewis, B. K.; Bryant, L. H.; Bulte, J. W. M. *Radiology* **2003**, *228*, 480–487.
(5) (a) Lombardo, L. J.; Lee, F. Y.; Chen, P.; Norris, D.; Sawyer, C. L. *J. Med. Chem.* **2004**, *47*, 6658–6661. (b) Shah, N. P.; Tran, C.; Lee, F. Y.; Chen, P.; Norris, D.; Sawyer, C. L. *Science* **2004**, *305*, 399–401.
(6) (a) Cifci, K.; Levy, R. *Int. J. Pharm.* **2001**, *218*, 81–92. (b) Fischer, R.; Köhler, K.; Fotin-Mleczek, M.; Brock, R. *J. Biol. Chem.* **2004**, *279*, 12625–12635.

JA8063843