Regulation of melanoma cell migration by pirin is revealed by a small molecule inhibitor

The discovery of small molecules that bind to a specific target and disrupt the function of proteins is an important step in chemical biology, especially for poorly characterized proteins. Chemical array methods enable us to isolate new ligands that bind to target proteins. Although proteins have large surface areas, many of the small-molecule ligands that have been identified in chemical array screens bind to functionally important sites in these proteins, thereby inhibiting their functions. Furthermore, many of these ligands presumably block protein-protein interactions, thereby affecting phenotypic and genetic changes in cells.

Human pirin is a nuclear protein widely expressed in punctate subnuclear structures in human tissues, but its function remains obscure. Pirin interacts with the nuclear factor I (NFI) [1] and Bcl-3 [2]. Pirin protein members are highly conserved between mammals, plants, fungi, and prokaryotic organisms and are considered to belong to the cupin superfamily. The cupin family is functionally diverse from every other protein family and comprises enzymatic and nonenzymatic members. Although the exact function of pirin is unknown, pirin orthologs have been reported to participate in many biological processes. There is evidence that the expression of human pirin is associated with the potential for cancer malignancies. The putative regulation of cancer cells by pirin is based on evidence that pirin binds to Bcl3, which is a proto-oncogene in chronic B-cell lymphocytic leukemia. Bcl3 interacts with the nuclear factor (NF)κB by binding to p50 and p52, and an increased Bcl3 expression level can enhance cell survival, proliferation, and tumor malignancy in many tumor cell lines.

A small molecule that binds specifically to pirin or proteins that interact with pirin would be a useful tool for determining the function of pirin, but no such molecule has been reported yet. To obtain pirin ligands, we performed a chemical array screen of mammalian cell lysates that overexpressed DsRed-fused proteins of interest. To obtain pirin binders, we screened more than 20,000 small molecules from a chemical library at the RIKEN Natural Products Depository (NPDepo) [3] using chemical arrays. From the screen, we isolated a small-molecule compound, which we named triphenyl compound A (TPh A; Fig. 2); it showed a positive binding signal on an array glass slide (Fig. 1). To confirm the specific binding signal from TPh A on the array slide and to study its structure-affinity relationship, we also spotted one structurally related compound onto the same slide (TPh B; Fig. 2). After the glass slide was incubated with cell lysates that expressed DsRed-fused pirin, an area that was spotted with TPh A generated a significant signal. By contrast, we found no significant signal in the area onto which TPh B was immobilized, relative to the background level.

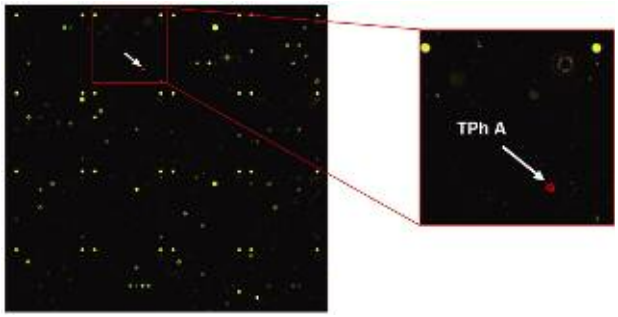

Fig. 1. The slide was treated with HEK293T cell lysates that overexpressed DsRed or DsRed-fused pirin. A fluorescent image is shown by merged display analysis of a glass slide. The positive signal of the area spotted with TPh A is indicated by white arrows.

To determine how TPh A binds to pirin, we solved the crystal structure of pirin complexed with TPh A at a 2.35-Å resolution using diffraction data collected at beamline **BL26B2**. Pirin is composed of two structurally similar β-barrel domains that are arranged

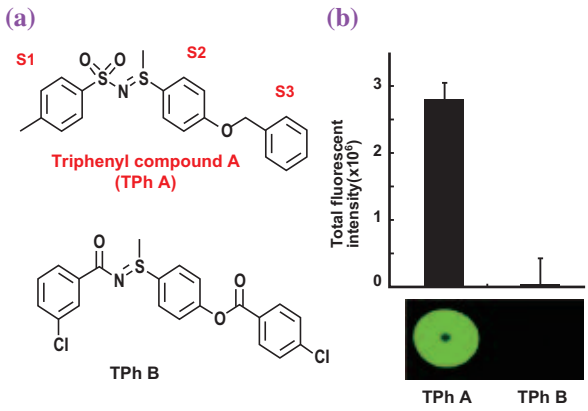

Fig. 2. **(a)** The structures of TPhs A and B are shown. TPh A has three phenyl groups (S1-S3) and a sulfilimine moiety. **(b)** TPhs A and B (10 mM) were immobilized onto a glass slide. The slide was incubated with HEK293T cell lysates that overexpressed DsRed-fused pirin. A fluorescent image is shown, and the total fluorescent intensity was corrected for background intensity. The error bars denote the s.d. of three replicates.

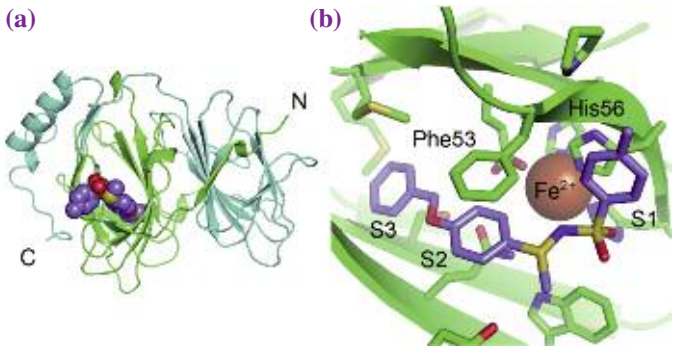

Fig. 3. **(a)** Overall view of crystal structure of pirin in complex with TPh A. Pirin is showed in green and cyan for the N- and C-terminal regions, respectively. The N- and C-terminal domains are cross-linked, with a single iron ion (brown) in the N-terminal domain. TPh A is shown as a CPK model. **(b)** TPh A molecule and the surrounding residues of pirin. The phenyl groups at the S1 and S2 positions of TPh A are stabilized by stacking interactions with His56 and Phe53, respectively.

face to face, with Fe^{2+} bound in the cavity of the N-terminal domain [4]. Our crystallography data revealed that TPh A binds in the cavity, where the metal binding site is found (Fig. 3(a)). The overall structure of the complex with TPh A resembled the structure without TPh A. The phenyl group in the S2 position of TPh A lay in the middle of the cavity, and this moiety interacted with Phe53 and the S1 ring interacted with the imidazole ring of His56 by a stacking effect (Fig. 3(b)). The metal-binding site, in which the Fe^{2+} was fixed by His56 and three coordinated sidechains, was exposed to two water molecules, one of which formed a hydrogen bond with the nitrogen atom in the *N*-(*p*-tolylsulfonyl)sulfilimine moiety of TPh A.

To gain insight into the effect of TPh A on cells, we measured the expression of pirin in several human cell lines. We found that pirin was highly expressed in the melanoma cell line WM266-4 (human malignant melanoma cells). In a screen of phenotypic changes in cells that were treated with TPh A, TPh A was found to have an effect on wound healing in WM266-4 cells, that is, the inhibition of cell migration was dose-dependent (Fig. 4). To determine whether knockdown of pirin affects melanoma cell migration, we treated cells with small inhibitory RNAs. The knockdown of pirin suppressed melanoma cell migration, as measured by wound-healing assay, but caused no change in cell proliferation.

We have identified an inhibitor of pirin, TPh A, and determined the role of pirin in melanoma cell migration. Furthermore, our crystallography data on the pirin-TPh A complex have revealed a small molecule-binding pocket in pirin, providing a new avenue through which we can gain a more thorough understanding of its structure and interactions with Bcl3 [5].

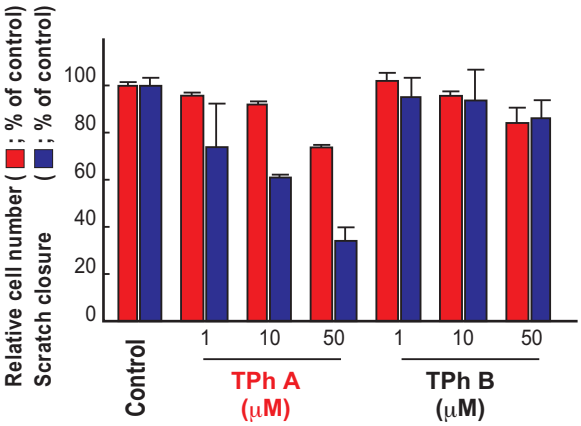

Fig. 4. Percentage of wound repair after 18 h in the absence or presence of various concentrations of TPh A or B (blue and red bars indicate cell proliferation and scratch closure, respectively). The relative cell number was calculated after 48 h (blue bars).

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