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Review

Proteomic identification of binding targets of isothiocyanates: A perspective on techniques

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ABSTRACT

Intake of cruciferous vegetable is inversely associated with the risk of several cancer types. Isothiocyanates (ITCs) are believed to be important constituents contributing to these cancer-preventive effects. Although several mechanisms, including induction of apoptosis, have been proposed for the anti-carcinogenesis activities of ITCs, detailed upstream triggering events are still not fully understood. Identification of ITC binding targets in cellular proteins is crucial for not only mechanistic studies but also future drug screening and design. In this review, we summarize recent progress in discovery of ITC protein targets from a technical perspective. The advantages and limitations of each method are discussed to facilitate future studies on target discovery of ITCs and perhaps other compounds.

Contents

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1. Introduction

Naturally occurring isothiocyanates (ITCs), including benzyl ITC (BITC), phenethyl ITC (PEITC), and sulforaphane (SFN), are effective cancer chemopreventive compounds [1,2]. Mounting evidence from animal studies indicates their anti-carcinogenic activity in all three major stages of tumor growth: initiation, promotion, and progression. Epidemiological studies have also shown that dietary intake of ITC is associated with reduced risk of human cancers [1,2]. Current literature indicates that induction of cell cycle arrest and apoptosis are important for the cancer preventive activity of ITCs [1–3]. However, the detailed mechanisms, especially the earliest interactions between ITCs and their molecular targets, are still unclear [4].

ITCs may react with nucleophilic amino acid residues in cellular proteins. The potential binding sites include thiol-containing cysteine, amine-containing lysines, arginine, and proline, and hydroxyl-containing serines, theronine and tyrosine. Among these sites, cysteines, especially the ionized cysteines (thiolate), are the strongest in proteins and represent the most likely binding sites of ITCs (Fig. 1) [5]. Cysteines, being nucleophilic, redox-active, and metal binding active, are both structurally and functionally important components in many enzymes [6]. Modifications of cysteine, through disulfide formation, oxidation, glutathionation, thioester formation, or direct covalent binding, may have critical biological consequences. For example, modification of reactive cysteines in Keap1 by SFN has been implicated as a trigger for Nrf2 activation [7]. Therefore, identification of binding targets of ITCs is a key step in elucidating mechanisms by which these compounds induce various downstream activities. This knowledge may also guide the screening and design of anti-cancer compounds with more efficacy and fewer side effects. However, the target identification is complicated and sometimes can be difficult due to multiple reasons. For example, modified proteins are likely to account for only a small fraction of the whole proteome. Searching for such proteins and pinpointing the exact site of modification can be as challenging as searching for a needle in a haystack. To track down a rare modification such as that by ITCs *in vivo* requires approaches with sufficient sensitivity and specificity. Also, ITC-modified proteins may undergo a series of transformations. For example, tubulin forms aggregates as a result of the structural misfolding induced by ITC modification and is consequently degraded through the ubiquitin-proteasome system in living cells [8,9]. As we discuss later, proteasome-mediated degradation may be a common event for several potential ITC target proteins.

Fortunately, the unique chemical property of ITCs and their rapid formation of covalent bond with potential targets allow us to trace and identify the modified proteins with the following approaches: First, target proteins were labeled with ITCs and identified using proteomic techniques; second, ITC-induced functional and structural changes of the target proteins were demonstrated using appropriate biological assays *in vitro* and *in vivo*; third, the ITC affinity to target proteins was determined and ITC binding sites in the target proteins confirmed. In this review, we summarize recent progress in ITC protein target discovery from a technical angle. Each method in different stages of the target identification is discussed in terms of advantage and disadvantage.

2. Protein target identification

Two proteomic techniques have been used in the discovery of ITC target proteins. The first approach is to label target proteins with radioactive ITCs in cultured cells and purify target proteins in the cell lysate using two-dimensional gel electrophoresis (2D-GE). The visualized radiolabeled proteins are located and identified by mass spectrometry (Fig. 2A). The other method is to "pull down" target proteins from cell lysate using affinity chromatography. This technique relies on the design of hybrid probes with ITC functional group at one end and a crosslinking probe at the other. The ITC functional group serves as the "bait" for target proteins while the crosslinker is required for facile and selective solid-phase target purification (Fig. 2B).

2.1. *Radio-labeling and two-dimensional gel electrophoresis*

Two-dimensional gel electrophoresis (2D-GE) has been frequently used to separate complex protein mixtures such as cell lysates. Mass spectrometry (MS) has become the premier technology for identifying proteins after 2D-GE. The combination of 2D-GE and MS has been used not only to reveal protein expression in cells [10] but also to identify drug targets. For example, ^{14}C-labeled acetaminophen has been used in target discovery in liver proteins [11,12]. Inspired by this pioneering work, we treated human non-small cell lung cancer A549 cells with 20 μM ^{14}C-PEITC or ^{14}C-SFN for 1 h [8]. The α-carbon in the phenethyl group was labeled as ^{14}C as a tracer for ITC-conjugated proteins (Fig. 3A). After treatment, cytoplasmic proteins were fractionated from the whole cell lysate and

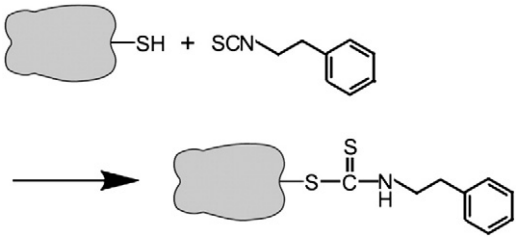

Fig. 1 – Thiocarbamation between PEITC and a protein cysteine.