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From protein–protein interaction to therapy response: Molecular imaging of heat shock proteins

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ABSTRACT

HSP70 promoter-driven gene therapy and inhibition of HSP90 activity with small molecule inhibitors are two shining points in a newly developed cohort of cancer treatment. For HSP70 promoters, high efficiency and heat inducibility within a localized region make it very attractive to clinical translation. The HSP90 inhibitors exhibit a broad spectrum of anticancer activities due to the downstream effects of HSP90 inhibition, which interfere with a wide range of signaling processes that are crucial for the malignant properties of cancer cells. In this review article, we summarize exciting applications of newly emerged molecular imaging techniques as they relate to HSP, including protein–protein interactions of HSP90 complexes, therapeutic response of tumors to HSP90 inhibitors, and HSP70 promoters-controlled gene therapy. In the HSPs context, molecular imaging is expected to play a vital role in promoting drug development and advancing individualized medicine.

1. Introduction

Molecular imaging is "the visualization, characterization, and measurement of biological processes at the cellular and molecular levels in humans and other living systems" [1]. Molecular imaging is a powerful new research tool that has only been made feasible by recent collaborative developments from diverse fields such as multiple image-capture techniques, cell and molecular biology, chemistry, medicine, pharmacology, medical physics, biomathematics and bioinformatics. Advanced multiple molecular imaging modalities have shed new light on a wide range of biomedical topics, including imaging specific cellular and molecular processes like gene expression or protein–protein interactions to optimize drug and gene therapies [2]. In this review, after a brief introduction of molecular imaging and heat shock proteins (HSPs), we discussed some important applications of molecular imaging for HSPs, mainly HSP70 and HSP90.

2. Molecular imaging

A molecular imaging agent typically has three main components: the targeting carrier, the imaging label, and a linker moiety (Fig. 1) although in some cases only one or two components also have the full function [3]. To date, a wide range of molecular imaging modalities are available, including techniques such as the positron emission tomography (PET), single-photon emission computed tomography (SPECT), molecular magnetic resonance imaging (mMRI), magnetic resonance spectroscopy (MRS), contrast enhanced ultrasound (CEU), optical bioluminescence, and optical fluorescence [2,4].

At present, PET is considered the most sensitive and specific technique for imaging molecular pathways *in vivo* in humans [5]. With probes labeled with positron-emitting radionuclides, PET produces a three-dimensional image or map of functional processes in the body. Another radionuclide-based imaging modality is SPECT. Due to its use of lead collimators to define the angle of incidence, however, SPECT imaging has a relatively low detection efficiency ($<10^{-4}$ times the emitted number of gamma rays) [6].

Non-radionuclide-based imaging techniques include MRI, ultrasound, and optical imaging. MRI is a non-invasive diagnostic technique based on the interactions of protons (or other nuclei) with one other and with surrounding molecules in a tissue of interest [7]. Different tissues have different relaxation times that can result in endogenous contrast. The major advantages of MRI over radionuclide-based imaging are the absence of radiation and higher spatial resolution (usually sub-millimeter level). The major disadvantage of MRI is its inherent low sensitivity, which can be partially compensated by working at higher magnetic fields (4.7–14 T), acquiring data for longer time periods during imaging, and using exogenous contrast agents. For ultrasound imaging, high-frequency sound waves are emitted from a transducer placed against the

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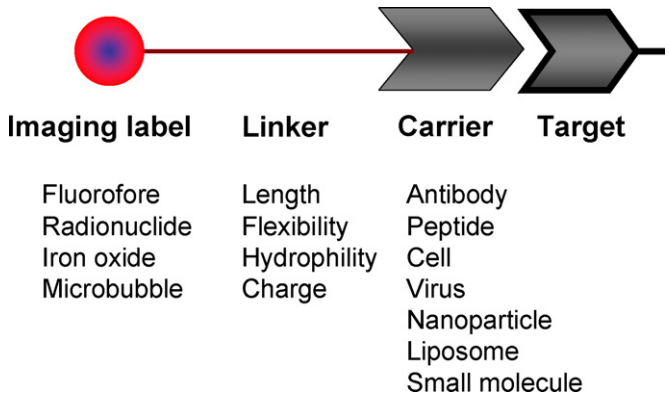

Fig. 1. An imaging agent is typically composed of an imaging label, a carrier that is/contains a targeting ligand, and a linker between the carrier and the label.

skin and ultrasound images are obtained based on the sound wave reflected back from the internal organs. The contrast of ultrasound is dependent on the sound speed, sound attenuation, backscatter, and the imaging algorithm [8]. Because of its safety, low cost, ease of use, and wide availability, ultrasonography is one of the most commonly used clinical imaging modalities [9]. Ultrasound contrast agents have been used in the clinic for applications such as blood pool enhancement, characterization of liver lesions, and perfusion imaging [10,11]. These contrast agents are generally in the form of small acoustically active particles ranging from several hundred nanometers to a few micrometers in diameter. X-ray computerized tomography (CT) is a medical imaging method that uses digital geometry processing to generate a 3-D image of the internals of an object from a large series of two-dimensional X-ray images taken around a single axis of rotation [12]. However, CT is not a typical molecular imaging modality due to the lack of target-specific contrast agents.

Two main branches of optical imaging are fluorescence imaging and bioluminescence imaging. In fluorescence imaging, excitation light illuminates the subject, and a charge-coupled device (CCD) camera collects the emission light at a shifted wavelength [2]. A number of high-resolution microscopic imaging techniques have recently been developed to study molecular events *in vivo*. In particular, intravital fluorescence microscopy [13], confocal laser scanning microscopy [14], multiphoton laser scanning microscopy [15], and *in situ* scanning force microscopy [16] have recently been introduced. The major limitation of optical imaging is that tissue light scattering and absorption can affect both image resolution and depth of light penetration of tissues [4]. Optical coherence tomography (OCT), an imaging technique with higher spatial resolutions (typically 10–15 μm), allows for real-time, cross-sectional imaging through biological tissues [17]. A series of fluorescent proteins, such as the green fluorescent protein, have also enabled sophisticated studies of protein function and wide-ranging processes from gene expression to second-messenger cascades and intercellular signaling, typically through fusion protein approach rather than through direct labeling [18]. Bioluminescence imaging (BLI) is based on the expression of a light-emitting enzyme (such as firefly luciferase) in target cells and tissues [19]. In the presence of its substrate (such as D-luciferin), an energy-dependent reaction releases photons that can be detected using sensitive detection systems. BLI has been applied for various applications such as studying gene-expression patterns [19], measuring gene transfer efficiency [20], monitoring tumor growth and response to therapy [21], investigating protein–protein interactions *in vivo* [22,23], and determining the location and proliferation of stem cells [24].

3. Heat shock proteins

Following exposure to protein-damaging environmental stresses, cells dramatically increase the expression of a group of proteins as an adaptive response to enhance protein renaturation, restore homeostasis, and improve survival—a process termed the "heat shock response" [25]. These heat shock proteins are named according to the approximate relative molecular weights of their encoded proteins, including HSP10, HSP27, HSP40, HSP60, HSP70, HSP90 and HSP110 [26,27]. The cytoprotective properties of the HSPs are closely linked to their primary functions as molecular chaperones [26].

HSP70 and HSP90 families are principal holding proteins which bind to unfolded sequences in polypeptide substrates and show preference for hydrophobic regions [28]. HSP70 and HSP90 function in large complexes or 'chaperone machines' containing several accessory proteins or co-chaperones that bind the primary chaperone to mediate substrate selection and cycles of association with, and disassociation from, the substrate [29]. After completion of their molecular chaperone function, HSP70 and HSP90 are actively released from protein substrates by means of their intrinsic ATPase domains [26].

In addition to serving as molecular chaperones, HSPs also have key functions in controlling cellular metabolism. Cell regulation by molecular chaperones is mediated by the holding ability of HSP70 and HSP90, each of which can bind stably to several regulatory molecules [29]. By interacting with key regulatory proteins, HSP90 has a principal role in regulating mitogenesis and cell-cycle progression, whereas HSP70 is closely involved in guarding against programmed cell death (PCD). Numerous cellular proteins undergo synchronous unfolding owing to the chaotropic effects of heat, which can lead to the cellular catastrophe of protein aggregation. Such protein aggregation is deterred by engagement of the heat-shock response and the accompanying abundant expression of the HSP cohort, which recognizes denatured proteins through the holding properties of HSP27, HSP70 and HSP90, and subsequently refolds them with the aid of chaperonins [30]. In addition, because protein denaturation and aggregation are powerful triggers of PCD, HSPs have developed powerful anti-apoptotic properties that deter PCD [29] and thus permit a time window for subsequent repair of the proteome [31]. The massive upregulation of HSPs that occurs during heat shock involves facilitation of expression at different levels, including activation of the potent heat-shock transcription factor 1 (HSF1), stabilization of HSP mRNA, selective translation of HSPs, and stabilization of HSPs at the protein level [32].

4. HSP90 and cancer

HSP90 is a key member of molecular chaperones that promote the proper folding of nascent polypeptides and ensure that protein–protein interactions occur in a productive manner under basal conditions [33]. HSP90 is an ATP-dependent molecular chaperone that forms a constitutive dimer. The N-terminal domain of HSP90 contains a unique ATP-binding site termed the Bergerat fold [34], which is essential for the ATP-dependent function of the chaperone *in vitro* and *in vivo*. Compared to other chaperones, HSP90 binds to substrate proteins that are at a later stage of folding [35]. Another unique characteristic of HSP90 is that it targets a specific set of client proteins that are mainly involved in signal transduction pathways including wild-type and mutated AR, p53, HER2, and Akt [29,36–38].

The HSP90 complex consist of the chaperone HSP70 and its co-chaperone HSP40, adapter protein Hop/p60, protein p23, and other helper proteins. The interactions between HSP90 and many of its co-chaperones occur within the C-terminal domain [29]. The dynamic interactions of HSP90 with its co-chaperones and client proteins