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Review

Strategies for detection and quantification of cysteine cathepsins-evolution from bench to bedside

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ABSTRACT

The cysteine cathepsins are a family of closely related thiol proteases, normally found in the endosomal and lysosomal compartments of cells. A growing body of evidence has clearly linked the dysregulated activity of these proteases with many diseases and pathological conditions, offering therapeutic, prognostic and diagnostic potential. However, these proteases are synthesised as inactive precursors and once activated, are controlled by factors such as pH and presence of endogenous inhibitors, meaning that overall protein and activity levels do not necessarily correlate. In order to fully appreciate the role and potential of these proteases, tools are required that can detect and quantify overall cathepsin activity. Two main strategies have evolved; synthetic substrates and protease-labelling with affinity-binding probes (or activity-based probes). This review examines recent innovations in these approaches as the field moves towards developing tools that could ultimately be used in patients for diagnostic or prognostic applications.

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1. Cysteine cathepsins in disease

Proteases are a class of enzymes that catalyse the hydrolysis of peptide bonds and therefore irreversibly modify the function of other proteins. As a result, proteases are implicated in a wide array of physiological processes which are essential for cellular homeostasis [1]. Proteases characterised to date can be broadly classified on the basis of their catalytic mechanism and key residues/ion in their active site. These include the metalloproteinases (MMPs), serine, threonine, aspartic, glutamic acid and cysteine proteases [2–4].

The cysteine cathepsins, also known as sulfhydryl or thiol proteases are members of the C1a family of clan CA proteolytic enzymes [3]. This class of protease is characterised by the presence of a cysteine residue within their active site, the thiol moiety of which acts as nucleophile towards the carbonyl carbon of the substrate scissile bond [5]. There are 11 members of the human cysteine cathepsin family including cathepsins B, C, H, F, K, L, O S, V, W and X [4]. These proteases were once thought to be solely involved in the non-specific terminal digestion of internalised proteins within lysosomes of cells [6]. However, several of these cathepsin genes exhibit individual expression patterns indicating specialised physiological roles, and over the last 10–15 years we have come to appreciate the involvement of these proteases in a range of both physiological and pathological roles [7–10] (Table 1).

2. Detection of cysteine cathepsin activity

Proteolytic cleavage is an example of a post-translational modification (PTM) towards a target protein (substrate), but unlike many other PTMs is generally irreversible. Therefore proteases tend to control key points in many biological processes and are frequently of clinical importance. Thus, it is unsurprising that proteolysis is strictly controlled *in vivo*. Indeed cysteine cathespins are firstly synthesised as precursor zymogens that require further proteolytic processing to become catalytically active [11]. Compartmentalisation and pH of the lysosomal lumen are also key determinants in controlling the activities of these enzymes [12,13], as are the presence of endogenous cognate inhibitors such as cystatins and PTMs including glycosylation [14,15]. Consequently, dysregulation of these control mechanisms can promote the role of cysteine proteases in various disease states, as shown in Table 1, highlighting their potential as therapeutic targets. However, these disease associations also reveal that these proteases may be useful biomarkers of such pathologies.

Given that cysteine cathepsins are subject to dynamic regulation and are stored within the cell in a precursor inactive state, the detection of proteases through methods which measure total protein levels (e.g., ELISA, real-time PCR or immunohistochemistry), will not necessarily correlate with activity; which could be a critical factor in determining their usefulness as biomarkers [16]. Consequently, the development of quantitative detection assays which are able to detect the functional state of cysteine cathepsins in their native environment are required.

The ability to visualise cathepsin activity could be a valuable tool in detecting a disease, determining extent of progression and informing therapeutic decisions [17]. For these reasons, interest in activity-based enzyme detection has soared in the last 15 years to create contrast agents or 'probes' which can report on the levels of functional cathepsins in complex proteomes.

The long-term goal of monitoring proteolytic activity would be to bring imaging tools into the clinic where they can report on molecular events in real-time, in a non-invasive manner. This review discusses the development and application of tools to detect cathepsin activity and highlights the creative approaches being applied to probe design to overcome the challenges associated with more complex *in vivo* imaging.

3. Quenched reporter substrates

The most widely used approach for the detection of proteolytic activity involves use of fluorescent contrast agents. This strategy for detecting proteolytic activity relies upon the use of fluorescent reporter substrates, which exploits Förster or fluorescence resonance energy transfer (FRET). FRET describes the energy transfer between a donor and acceptor fluorophore pair, creating an internally quenched reporter substrate (Fig. 1A), which is only activated upon proteolytic cleavage and separation of the fluorophore/quencher pair (Fig. 1B) [18,19]. Conceptually, a donor fluorophore group is attached to an amino acid residue on one side of a scissile peptide bond and an acceptor fluorophore is attached to the other terminus of the sequence.

Table 1
Differential expression patterns of cathepsins and their involvement in various diseases.

Name	Chromosomal location	Expression pattern in tissue	Involvement in disease	Refs
Cathepsin **B**	8p22	Ubiquitous	Inflammatory disorders, rheumatoid arthritis, cancer	[136–138]
Cathepsin **C**	11q14.1–14.3	Ubiquitous	Papillon-Lefevre syndrome, Haim-Munk syndrome	[139,140]
Cathepsin **F**	11q13	Macrophages	Cancer, Kufs disease	[141,142]
Cathepsin **H**	15q24-25	Ubiquitous	Cancer, pulmonary disorders, Myobia	[143–145]
Cathepsin **K**	1q21	Osteoclasts, bronchial epithelium	Atherosclerosis, rheumatoid arthritis, Osteoporosis, Pycnodysostosis	[8,146,147]
Cathepsin **L**	9q21-22	Ubiquitous	Atherosclerosis, cancer, abdominal aortic aneurysm	[148,149]
Cathepsin **O**	4q31-32	Ubiquitous	Protein degradation and turnover	[150,151]
Cathepsin **S**	1q21	Antigen presenting cells	Cancer, atherosclerosis, obesity, emphysema, rheumatoid arthritis	[127,152–156]
Cathepsin **V**	9q22.2	Thymus epithelium	Thymic pathology, cancer	[157,158]
Cathepsin **W**	11q13.1	CD8+ T cells	Autoimmune atrophic gastritis	[159]
Cathepsin **X/Z**	20q13	Ubiquitous	Cancer	[160]

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