Lizosomalni katepsini in njihovi endogeni inhibitorji − 50 let raziskav

Vito Turk
Institut Jožef Stefan & MPŠ

Historical Milestones

- 1928: Term cathepsin introduced by R. Wilstatter & E. Bamann
- 1937: First purification of cathepsin by M.L. Anson
- 1952-1960: Several cathepsins purified and characterized
- 1955: Discovery of lysosome by C. deDuve
- 1983: First a.a sequences of: a) mammalian cathepsins B and H by N. Katunuma’s group; b) rat cystatin β (Katunuma’s group) and human stefin B (V. Turk’s group)
- 1988-1990: X-ray crystal structures of chicken cystatin and human stefin B revealed mechanism of interaction with papain-like enzymes (cathepsins)
- 1990-: Genomics, proteomics, degradomics for unravelling biology and medicine
INTACT LYSOSOMES
ENZYMES BOUND AND ACCESSIBLE

Waring blender

INJURED LYSOSOMES
ENZYMES SOLUBLE AND ACCESSIBLE
(5 acid hydrolases incl. Cath D)

Discoverer of lysosome (1955) visiting

Photograph courtesy of
Marjan Smerke

The function of lysosomes
C. De Duve (1963)

The four digestive processes mediated by the lysosome
A. Ciechanover (2005, 2012)
“The history of proteolytic enzymes is intimately intertwined with that of protein chemistry. In the very yearly days, proteolytic enzymes were considered an impediment that had to be removed in the isolation of proteins generally.

...Interest in proteases was considerably stimulated by the recognition that, aside from their digestive action, proteases are involved in the regulation of great many physiological processes. ... The term “limited proteolysis” was coined by Linderstrom-Lang to differentiate the restricted specificity of certain enzymes under certain conditions from the random proteolysis accompanying protein degradation. Proteolytic processing can be limited by the specificity of the protease, the obligatory activation of an ENZYME PRECURSOR, the action of PROTEASE INHIBITORS, or a combination of these factors.... Proteases can be grouped into families of common mechanism, similar structural features, and hence common evolutionary origin. They included the well known families of serine, CYSTEINE, aspartic, and metallo endo- and exopeptidases.”

Current numbers of peptidases in humans and other species

<table>
<thead>
<tr>
<th>Species</th>
<th>Total*</th>
<th>Aspartic</th>
<th>Cysteine</th>
<th>Metallo</th>
<th>Serine</th>
<th>Threonine</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Homo sapiens</em></td>
<td>461</td>
<td>18</td>
<td>121</td>
<td>159</td>
<td>140</td>
<td>23</td>
</tr>
<tr>
<td><em>Caenorhabditis elegans</em></td>
<td>353</td>
<td>26</td>
<td>93</td>
<td>151</td>
<td>62</td>
<td>21</td>
</tr>
<tr>
<td><em>Drosophila melanogaster</em></td>
<td>513</td>
<td>38</td>
<td>59</td>
<td>157</td>
<td>225</td>
<td>34</td>
</tr>
<tr>
<td><em>Mus musculus</em></td>
<td>383</td>
<td>11</td>
<td>93</td>
<td>120</td>
<td>137</td>
<td>22</td>
</tr>
<tr>
<td><em>Rattus norvegicus</em></td>
<td>227</td>
<td>10</td>
<td>41</td>
<td>77</td>
<td>80</td>
<td>19</td>
</tr>
</tbody>
</table>

*The total numbers are from MEROPS database version 5.7: http://www.merops.ac.uk*
Cathepsins

• Any of various proteolytic enzymes found in animal tissue that catalyze the hydrolysis of proteins into polypeptides.
• [German: Kathepsin, from Greek kathepsein, to digest: kat-, kata-, cata- + hepsein, to boil; English: Cathepsin]

• Serine cathepsins: A, G
• Aspartic cathepsins: D and E
Papain-like (cysteine) cathepsins

- building blocks:
  **signal peptide-propeptide-mature protease**
- active and stable at acidic pH
- **Endopeptidases:** cathepsins L, V, S, K, F, W?, B, H
- **Exopeptidases:**
  - **carboxyopeptidases:**
    - cathepsins B and X
  - **aminopeptidases:**
    - cathepsins C and H
CATHEPSIN L - endopeptidase
CATHEPSIN L - endopeptidase

CATHEPSIN H - aminopeptidase
CATHEPSIN L - endopeptidase

CATHEPSIN H - aminopeptidase

CATHEPSIN X - carboxypeptidase
Substrate binding sites

Physiological and pathophysiological role of cysteine cathepsins and their protein inhibitors

- Intracellular protein degradation
- Antigen processing and presentation (cathepsins S, L, V, F, cystatin C)
- Bone remodelling (cathepsin K, cathepsin L)
- Protein processing (cathepsin B, S, C…)
- Apoptosis (cathepsin C, B, L, K, S…)

- Cancer (cathepsin B, H, L, S, F …)
- Rheumatoid arthritis and osteoarthritis (cathepsin B, K, S)
- Osteoporosis (cathepsin K, L)
- Cardiovascular diseases (cathepsin B, L, S…)
- Periodontitis (cathepsin C)
- Skin disorders (cathepsin C, stefins)
- Lung emphysema (cathepsin L)
- Neurodegeneration (cathepsin S, B, L, K, …, stefin B, cystatin C)
Genetic diseases involving cysteine cathepsins and their protein inhibitors

- hereditary cystatin C amyloid angiopathy (HCCAA)
- progressive myoclonus epilepsy (EPM 1) - stefin B
- Papillon-Lefévre syndrome, Haim-Munk syndrome (also keratosis palmoplantaris) – cathepsin C
- Pycnodysostosis – cathepsin K
Propeptides of papain-like cysteine proteases

Cathepsin L like prosegment
(L,F,K,H,S,V, W)
• ~ 100 residues
• ERF(W)NIN and GNFD motif

Cathepsin B like prosegment
• 60 residues
• GNFD motif only

Cathepsin C, O, X
-prosegment of different size
Sequence alignment of human cysteine cathepsins
Cathepsin B

- single chain of 248 residues
- ~20 amino acids long insertion - occluding loop
- dipeptidyl carboxypeptidase and endopeptidase
Cathepsin H

- aminopeptidase with broad substrate specificity (Phe-, Trp-, Arg-, Leu-, Lys-, Ala-)
- single or two-chain enzyme, glycosylated
- additional octapeptide (mini-chain) $EPQNCSAT$, S-S bound to the main body
- crystal structure
Cathepsin C (DPPI)

• amino- dipeptidyl peptidase I (DPPI)
• no endopeptidase activity
• tetrameric structure
• additional exclusion domain with Asp 1 residue anchoring the N-terminal of the substrate
• physiological activator of serine proteases from granules of immune (cytotoxic T-lymphocytes, natural killer cells) and inflammatory (neutrophils, mast cells): granzymes A, B and C, cathepsin G, neutrophil elastase and chymase
Cathepsin C (DPPI) tetramer
Mutations of DPPI resulting in genetic diseases
Y 323 C mutation of DPPI

- disrupts chloride ion binding, positioning of Phe 278 and Asp 1
- possible new disulphide bridge to Cys 331
- disrupted binding surface for exclusion domain • no oligomeric structure • endopeptidase activity
Keratosis Palmoplantaris
Cathepsins X, H, B and C superimposed on papain surface
Principles of protease activity regulation

- **Zymogen activation**
  - Propeptide is removed during processing
  - Function of propeptide
    - Targeting
    - Folding
    - Inhibition
    - Stability

- **Cysteine protease inhibitors**
  - Cystatins (Stefins, Cystatins, Kininogens) and other related proteins (CRES, tick cystatins etc)
  - Thyropins
Inhibitors of papain-like cysteine peptidases

- **Cystatins**
  - stefins (stefin A, stefin B, + variants)
  - cystatins (classical: C, D, E/M, F, S, SA, SN; possibly cystatin 10, crestatins: cys T, testatin, cys SC, CRES, cystatin 11 (CRES 2 in mouse), CRES3)
  - kininogens (LK, HK, TK)
- **Thyropins** (p41 li fragment, equistatin, saxiphilin, ECI, testican, …)
- **Serpins** (SCCA1, endopin 2, MENT, hurpin, Spi A2, SQN 5, …)
- **beta-barrel type inhibitors**
  - lipocalins (tear lipocalcin, staphostatin B, …)
  - cat C exclusion domain
- **propeptides** (propeptides, cytotoxic T-lymphocyte antigen 2b)
- **alpha₂-macroglobulin**
Alignment of stefins, cystatins and kininogens

<table>
<thead>
<tr>
<th>Type 1</th>
<th>Stefin A</th>
<th>1</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>70</th>
<th>80</th>
<th>90</th>
<th>100</th>
<th>110</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stefin B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type 2</th>
<th>Cystatin S</th>
<th>1</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>70</th>
<th>80</th>
<th>90</th>
<th>100</th>
<th>110</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystatin SA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cystatin SB</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type 3</th>
<th>Kininogen 2</th>
<th>1</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>70</th>
<th>80</th>
<th>90</th>
<th>100</th>
<th>110</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kininogen 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CRES* subgroup of cystatins

* Cystatin Related Epididymal Spermatogenic (CRES) proteins
The 2.0 Å X-ray crystal structure of chicken egg white cystatin and its possible mode of interaction with cysteine proteinases
Human Stefin B: polypeptide fold

Stubbs et al. (1990) EMBO J. 9: 1939
The refined 2.4 Å X-ray crystal structure of recombinant human stefin B in complex with the cysteine proteinase papain: a novel type of proteinase inhibitor interaction.

Cathepsin L-p41 fragment complex

<table>
<thead>
<tr>
<th></th>
<th>Gly 139</th>
<th>Glu 159</th>
<th>Asp 160</th>
<th>Asp 162</th>
<th>Glu 141</th>
<th>Leu 144</th>
</tr>
</thead>
<tbody>
<tr>
<td>cathepsin L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cathepsin S</td>
<td>Arg 141</td>
<td>Gln 160</td>
<td>Asn 161</td>
<td>Asn 163</td>
<td>Pro 143</td>
<td>Phe 146</td>
</tr>
</tbody>
</table>

Antigen processing and presentation

intracellular antigens → proteasome → antigenic peptides + MHC I → T killer cells

extracellular antigens → endosomal proteases → antigenic peptides + MHC II → T helper cells
Thyropins: Schematic diagram of Tg type-1 repeats

- Tg
- Nido asc
- Nido
- Sax
- GA733
- Testican
- p41
- IGFBP5
- ECI
- Equistatin

Legend:
- Orange: signal
- Red: Tg type-1 domain
- Gray: 100 amino acids
When a protease is druggable by a small compound?

- Only when its activity is higher under pathological conditions or it is found in an inappropriate compartment
  - Increased expression or activation of a protease
  - Change of subcellular localization
  - Decrease in regulatory inhibitor activity

- Not suitable for decreased activity of proteases such as in a number of genetic disorders
PROTEIN STRUCTURE BASED DRUG DESIGN CYCLE
Steps in drug discovery

• Target validation
• Target cloning, expression and assay development
• Inhibitor design, synthesis and screening (repeat if necessary); crystal structure is of great help! - Or alternatively, starting from small libraries of selected compounds (10000-25000)
• Inhibitor testing for selectivity (target vs. related proteases + antitargets) – go back if necessary
• LEAD COMPOUND (optimization)
• Compound chemical stability
• Pharmacokinetics and pharmacodynamics (at least two animal models)
• EARLY DRUG CANDIDATE
• Clinical studies (Phase I –III)
• If all OK - DRUG
Small molecule inhibitor design

- **Golden standard: reversible noncovalent inhibitors** – potentially the best due to high selectivity and least adverse effect, difficult to design
- **Covalent reversible inhibitors** – most often used, relatively successful, some selectivity problems (off-target effects) potentially immunogenic
- **Irreversible inhibitors** - probably not suitable for long-term treatment of chronic diseases, potential for short-term treatment of acute diseases – major problem selectivity and off-target effects, immunogenic
Epoxy-succinyl inhibitors

E64

CA030

CLIK066
Cathepsin B complexed with epoxysuccinyl based inhibitor (CA030)
Lessons from unsuccessful experiments:

• Good inhibitor usually does not make a good drug
• However, good inhibitors can be important reagents in elucidating protease functions in experimental systems (if you get them from pharma industry)
• Inhibitors of other proteases than the target protease can be obtained by screening
• Good assays are/can be developed
Pathophysiological function

- **hu-cystatin C** is well-known amyloidogenic protein
- co-deposition with Aβ in plaques of Alzheimer's disease
- some allelic variants increase the risk for Alzheimer's disease
- it causes cystatin C **amyloid angiopathy (CCAA)**, the hereditary form occurs if mutation L68Q. no plaques were found in pure CAAA, just haemorrhage

- **hu-stefin A**
- co-deposition with AD plaques
Cystatin C fold

Monomer

3D-domain swapped dimer

How to address biological functions of cathepsins?

- Tools development – activity-based probes, targeted delivery systems
- Proteomics – substrate identification
- Animal KO and transgene models; orthotopic transplantation
Imaging cathepsins and other proteases – a key to diagnostics, signaling pathways and target and compound validation

- Cathepsins highly suitable as they are often overexpressed in various diseases (good signal-to-noise ratio)
- Cathepsins in these diseases are often secreted from infiltrated immune cells – extracellular cathepsins markers of inflammation and inflammation-associated diseases
Lysosomal cathepsins and cancer

Vasiljeva & Turk (2008) Biochimie
Bid cleavage by lysosomal proteases

Targeted delivery systems: multifunctional tools (MRI-visible ferriliposomes)

- SPIO based nanoparticles
- Phospholipides
- PEG

- Hydrophilic compounds
- Lipophilic compounds

Improved anti-tumour effect of JPM-565 inhibitor

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment schedule (days after tumour volume reached 125 mm³)</th>
<th>Magnet</th>
<th>JPM-565</th>
<th>Liposome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>FLt</td>
<td>↓</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>JPM</td>
<td>↓</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>JPM+FL</td>
<td>↓</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>JPM+FLt</td>
<td>↓</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Improved anti-tumour effect of JPM-565 inhibitor

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment schedule (days after tumour volume reached 125 mm³)</th>
<th>Magnet</th>
<th>JPM-565</th>
<th>Liposome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>- - - - - - - - - - - - - - - - - - - - - - - - - - - - - -</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>FLt</td>
<td>↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>JPM</td>
<td>↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>JPM+FL</td>
<td>↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>JPM+FLt</td>
<td>↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

**Mikhaylov et al., (2011) Nature Nanotechnology**
What is protease signaling?

- Irreversible way of signal transduction, where signal is transmitted through cleavage of protein substrates
- Proteolytic processing is one of the most common posttranslational modifications of proteins

Challenges in protease signaling

- Where is the point of no return in protease signaling?
- Does a protease need to be active all the time?
- Can a temporarily active processed substrate transmit the signal?

Turk et al., 2012, EMBO J.
Identification of protease substrates is a key towards understanding protease signaling
Cleavage site specificity for cathepsins K, L and S

Vizovišek et al., *Proteomics* 2015
Perspectives in the field

• Unravelling biology (signaling pathways and substrates) in inflammation-associated diseases involving cathepsins (and other proteases)
• Biomarker identification and diagnostics
• Activity-based probes: signaling pathways, diagnostics, target identification, compound validation (reverse design, …)
• Targeted delivery systems for drug delivery and imaging: diagnostics and/or therapy (theranostics); compound validation, signaling pathways
• Proteases as *in vivo* tools to activate prodrugs or drug conjugates
Drug development is long and expensive

Developing a new medicine takes an average of 10–15 years; the Congressional Budget Office reports that “relatively few drugs survive the clinical trial process.”

Drug Discovery Preclinical Clinical Trials FDA Review Scale-Up to Mfg. Post-Marketing Surveillance

PRE-DISCOVERY

Drug Discovery

~ 5,000 – 10,000 COMPOUNDS

3 – 6 YEARS

Preclinical

250

5

4 – 6 YEARS

Clinical Trials

PHASE 1

PHASE 2

PHASE 3

NUMBER OF VOLUNTEERS

20–100

100–500

1,000–5,000

6 – 7 YEARS

FDA Review

Scale-Up to Mfg.

ONE FDA-APPROVED DRUG

0.5 – 2 YEARS

Post-Marketing Surveillance

INDEFINITE

<table>
<thead>
<tr>
<th>Protease inhibitors approved for clinical use or in clinical testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>• ACE (angiotensin converting enzyme) hypertension, cardiovascular disease, myocardial infarction</td>
</tr>
<tr>
<td>• HIV protease AIDS</td>
</tr>
<tr>
<td>• Thrombin thrombosis, stroke</td>
</tr>
<tr>
<td>• Factor Xa thrombosis</td>
</tr>
<tr>
<td>• Human neutrophil elastase respiratory disease</td>
</tr>
<tr>
<td>• MMP periodontitis</td>
</tr>
<tr>
<td>• Trypsin pancreatitis, inflammation</td>
</tr>
<tr>
<td>• Proteasome cancer</td>
</tr>
<tr>
<td>• DPP IV diabetes</td>
</tr>
<tr>
<td>• Renin hypertension, heart failure, obesity</td>
</tr>
<tr>
<td>• Factor IXa thrombosis</td>
</tr>
<tr>
<td>• <strong>Cathepsin K</strong> osteoporosis</td>
</tr>
<tr>
<td>• Elastase inflammation, psoriasis</td>
</tr>
<tr>
<td>• Caspase 1 (ICE) inflammation, arthritis</td>
</tr>
<tr>
<td>• Tryptase (beta) inflammation, asthma</td>
</tr>
<tr>
<td>□ γ-secretase Alzheimer disease</td>
</tr>
<tr>
<td>• MMPs cancer, rheumatoid arthritis</td>
</tr>
<tr>
<td>• Urokinase cancer</td>
</tr>
<tr>
<td>• TACE cancer, arthritis</td>
</tr>
<tr>
<td>• Factor VIIa thrombosis</td>
</tr>
<tr>
<td>• Histone deacetylase cancer</td>
</tr>
<tr>
<td>• <strong>Cathepsin S</strong> rheumatoid arthritis, psoriasis</td>
</tr>
<tr>
<td>□ □ □ □ □ □</td>
</tr>
<tr>
<td>• HCV NS3 protease hepatitis C (viral infection)</td>
</tr>
<tr>
<td>• Rhinovirus 3C protease viral infection</td>
</tr>
</tbody>
</table>
Za vsako bolezen obstaja vedno več kot ena tarča
Za vsako bolezen obstaja vedno več kot ena tarča

- Vprašanje za milijardo $: katera je najboljša?
Za vsako bolezen obstaja vedno več kot ena tarča

- Vprašanje za milijardo $: katera je najboljša?
- Vrhunsko znanje - ekscelentnost!
Statistični Urad Republike Slovenije R&D (Nov. 7, 2014)

Skupna poraba sredstev v vseh sektorjih R&D: 2,59% BDP

- 715,5 M EUR industrija (oz. 77%)
- 121,7 M EUR vladni sektor (13%)
- 97,0 M EUR visokošolski sektor (10%)
- 0,4 M EUR privatni neprofitni sektor
- Skupno 934,6 M EUR

Nekateri podatki istega Urada se razlikujejo npr.

- Vladni proračun 174,5 M EUR
- EU strukturno fondi 92,7 M EUR
- Skupno 267,2 M EUR ali 0.74% BDP ali 54,6 M EUR manj kot 2012.

- ARRS 2009: 184 M EUR (~0.8% BDP)
- ARRS 2014: 142 M EUR (0.48% BDP)
- ARRS 2015: 135 M EUR
UNESCO (Institut za statistiko 2014): %BDP v R&D

• Avstrija 2,84
• Danska 2,98
• Finska 3,55
• Nemčija 2,92
• Izrael 3,93
• Slovenija 2,80
• Švedska 3,41
• ZDA 2,79

Podatki IMF za Slovenijo 2014, %BDP
43 Miljard EUR, kar je 0.31% BDP za R&D
(dno Evrope, nerazvite države!!)

Zaključek
• Nujno je potrebno pregledati kaj predstavljajo industrijska vlaganja v R&D.
• Glede na ta vlaganja smo praktično v svetovnem vrhu in je ne razumljivo da imamo nizko dodano vrednost na proizvode in tako nizke plače v vseh sektorjih.
V razmislek o naši prihodnosti


Izjava YAE (Januar 2015)
“Bazične raziskave so na čelu moderne kulture: pomagajo nam razumeti kdo smo“
Acknowledgements

- Dept. Biochem. & Mol. & Struct. Biology, J. Stefan Institute, Ljubljana
  - Drago Lebez
  - Igor Kregar
  - Franc Gubenšek
  - Tamara Lah
  - Janko Kos
  - Majda Kopitar
  - Metka Renko
  - Anka Ritonja
  - Lojze Suhar
  - Vladimir Cotič
  - Tatjana Popovič
  - Jože Brzin
  - Marinka Drobnič-Košorok
  - Dušica Gabrijelčič
  - Brigita Lenarčič
  - Iztok Dolenc
  - Mojca Trstenjak
  - Roman Jerala
  - Veronika Stoka
  - Eva Žerovnik
  - Nataša Kopitar
  - Vida Puizdar
  - Ivica Štefe
  - Marko Dolinar
  - Tadeja Bevec
  - Marjeta Podobnik
  - Gregor Günčar
  - Robert Kuhelj
  - Galina Pungerčič
  - Dušan Kordič
  - Borut Štrukelj
  - Ana Petelin
  - Aleš Špes
  - Barbara Sobotič
  - Matej Vizovišek
  - Miha Butinar
  - Jelena Rajkovič
  - Jerica Rozman
  - Urška Repnik
  - Olga Vasiljeva
  - Georgy Mikhailov
  - Marko Fonovič
  - Dejan Čaglič
  - Gregor Kosec
  - Dušan Turk
  - Boris Turk

- Ludwig Maximilian University of Munich, Germany
  - Hans Fritz and coworkers

- Max-Planck Institute, Martinsried, Germany
  - Robert Huber and coworkers
  - Wolfram Bode

- University of Tokushima & Tokushima Bunri University, Japan
  - Nobuhiko Katunuma and coworkers

- DuPont de Nemours, Wilmington, USA
  - Bruce Korant
  - The Burnham Institute, La Jolla, USA
  - Guy Salvesen
  - Albert Ludwigs University, Freiburg, Germany
  - Christoph Peters
  - Thomas Reinheckel

- VIB, Ghent, Belgium
  - Kris Gevaert and coworkers

- Instituto de Investigaciones Biotecnologicas, Buenos Aires, Argentina
  - Juan Jose Cazzulo and coworkers

- Sanofi Aventis, Germany
  - Ulrich Wendt and coworkers

- Stanford University, USA
  - Matt Bogyo

- Tomsk Scientific Center RAS, Russia
  - Sergey G. Psakhye and coworkers

Financial support

MVZT, ARRS, NSF, VolkswagenStinftung, Kernforschungsanlage Jülich, FP4, FP7 etc.