Understanding and hijacking the oncogenic signalling adaptor proteins CRKL, Gab2 and Frs2

Stefano GIANNI
RESEARCH AREA: Genetica, biologia e fisiopatologia molecolare-cellulare degli Eucarioti

Receptor tyrosine kinase (RTK) signaling plays key roles in cell physiology and development. Impaired activation of these signaling pathways is critical in the genesis and progression of many types of cancers. RTK signaling pathways are generally activated by growth factor binding to a specific trans-membrane receptor, which activates downstream signaling molecules. These mechanisms require the formation of specific protein complexes mediated by adaptor proteins. Thus, adaptor proteins, while lacking any enzymatic activity provide a critical scaffolding function that facilitates key signaling transduction events and regulates signal specificity and amplification. Among these adaptors, three proteins, namely CRKL, GAB2, and FRS2, represent particularly interesting targets, being recurrently amplified in several types of cancers and essential to cancer cell lines that harbor such amplification. The overexpression of these three proteins is able to transform immortalized human cell lines in in vitro or in vivo models and their knockdown significantly reduces cancer proliferation. Based on these observations, we propose that an effective chemotherapeutic strategy would be that of interfering with CRKL, GAB2 and FRS2 protein-protein interaction network.

To provide a detailed structural characterization of the interactions between CRKL, GAB2 and FRS2 and thier partners, we focused on the interactions between these proteins and p85, Grb2 and SHP2. Therefore we successfully cloned, expressed and purified the N- and C-terminal SH2 domains from p85, the SH3 domain from Grb2 and the N- and C-terminal SH2 domains from SHP2. Subsequently, these constructs were subjected to NMR characterization both in the presence and in the absence of a peptide mimicking GAB2 , which allowed us to assign the structural changes induced by binding. Furthermore, we conducted an extensive biophysical analysis on these proteins domains, which allowed us to describe the mechanism of recognition of these proteins at nearly atomic resolution.

Finally, the research group was also involved in the characterization of the folding of several protein domains, with particular emphasis on the role of intradomain communication in multidomain proteins.

Publications

1) Malagrinò F, Pennacchietti V, Santorelli D, Pagano L, Nardella C, Diop A, Toto A, Gianni S. ***On the Effects of Disordered Tails, Supertertiary Structure and Quinary Interactions on the Folding and Function of Protein Domains*** Biomolecules. 2022 Jan 26;12(2):209. doi: 10.3390/biom12020209

I.F. 6.064

2) Malagrinò F, Diop A, Pagano L, Nardella C, Toto A, Gianni S. ***Unveiling induced folding of intrinsically disordered proteins - Protein engineering, frustration and emerging themes*** Curr Opin Struct Biol . 2022 Feb;72:153-160. doi: 10.1016/j.sbi.2021.11.004.

I.F. 7.786

3) Coluccia A, Bufano M, La Regina G, Puxeddu M, Toto A, Paone A, Bouzidi A, Musto G, Badolati N, Orlando V, Biagioni S, Masci D, Cantatore C, Cirilli R, Cutruzzolà F, Gianni S, Stornaiuolo M, Silvestri R. ***Anticancer Activity of (S)-5-Chloro-3-((3,5-dimethylphenyl)sulfonyl)- N-(1-oxo-1-((pyridin-4-ylmethyl)amino)propan-2-yl)-1 H-indole-2-carboxamide (RS4690), a New Dishevelled 1 Inhibitor*** Cancers (Basel). 2022 Mar 7;14(5):1358. doi: 10.3390/cancers14051358.

I.F. 6.575

4) Toto A, Malagrinò F, Nardella C, Pennacchietti V, Pagano L, Santorelli D, Diop A, Gianni S. ***Characterization of early and late transition states of the folding pathway of a SH2 domain*** Protein Sci. 2022 Jun;31(6):e4332. doi: 10.1002/pro.4332.

I.F. 6.993

5) Brunori M, Gianni S. ***An Outlook on the Complexity of Protein Morphogenesis in Health and Disease.*** Front Mol Biosci. 2022 Jun 13;9:909567. doi: 10.3389/fmolb.2022.909567.

I.F. 6.113

6) Nardella C, Toto A, Santorelli D, Pagano L, Diop A, Pennacchietti V, Pietrangeli P, Marcocci L, Malagrinò F, Gianni S. ***Folding and Binding Mechanisms of the SH2 Domain from Crkl.*** Biomolecules. 2022 Jul 22;12(8):1014. doi: 10.3390/biom12081014.

I.F. 6.064

7) Malagrinò F, Fusco G, Pennacchietti V, Toto A, Nardella C, Pagano L, de Simone A, Gianni S. ***Cryptic binding properties of a transient folding intermediate in a PDZ tandem repeat.*** Protein Sci. 2022 Sep;31(9):e4396. doi: 10.1002/pro.4396.

I.F. 6.993

8) Gautier C, Gianni S. A short structural extension dictates the early stages of folding of a PDZ domain. Biochim Biophys Acta Proteins Proteom. 2022 Nov 1;1870(11-12):140852.
I.F. 4.125

9) Pagano L, Pennacchietti V, Diop A, Santorelli D, Pietrangeli P, Marcocci L, Nardella C, Malagrinò F, Toto A, Gianni S. ***Exploring the effect of tethered domains on the folding of Grb2 protein.*** Arch Biochem Biophys. 2022 Nov 30;731:109444.
I.F. 4.114

10) Hollås H, Ramirez J, Nominé Y, Kostmann C, Toto A, Gianni S, Travé G, Vedeler A. ***The cooperative folding of annexin A2 relies on a transient nonnative intermediate.*** Biophys J. 2022 Dec 6;121(23):4492-4504.
I.F. 4.033

11) Diop A, Santorelli D, Malagrinò F, Nardella C, Pennacchietti V, Pagano L, Marcocci L, Pietrangeli P, Gianni S, Toto A. ***SH2 Domains: Folding, Binding and Therapeutical Approaches.*** Int J Mol Sci. 2022 Dec 15;23(24):15944.
I.F. 6.208

Research Group

Angelo Toto Researchers;
Caterina Nardella, Post Doc
Daniele Santorelli, Post Doc
Livia Pagano, PhD student
Awa Diop, PhD student
Valeria Pennacchietti, PhD student

Collaborations (if other than Pasteur)

Per Jemth, Uppsala University