Degree in Biochemistry

Universitat Autònoma de Barcelona

Guillem Prats Ejarque

RIBONUCLEASES AND AUTOPHAGY. RIBONUCLEASES AS ANTITUMORAL AGENTS

Introduction

Autophagy

- Dual role of autophagy. Pro-survival and pro-death[1].
- Autophagic cell death (Programmed Cell Death type II). Autophagy inhibitors rescue the cell from death.
- Promising new pathway to develop antitumoral drugs.

Antitumoral ribonucleases

- Members of the RNase A superfamily. Small and cationic secretion ribonucleases.
- Degradation of the cell RNA triggers cell death.
- They enter specifically cancer cells due to the anionic properties of their membrane.
- Autophagic cell death has been reported to be induced by the two main antitumour ribonucleases[2,3,4].
  - Bovine Seminal RNase (BS-RNase)
  - Bullfrog RNase (onconase)

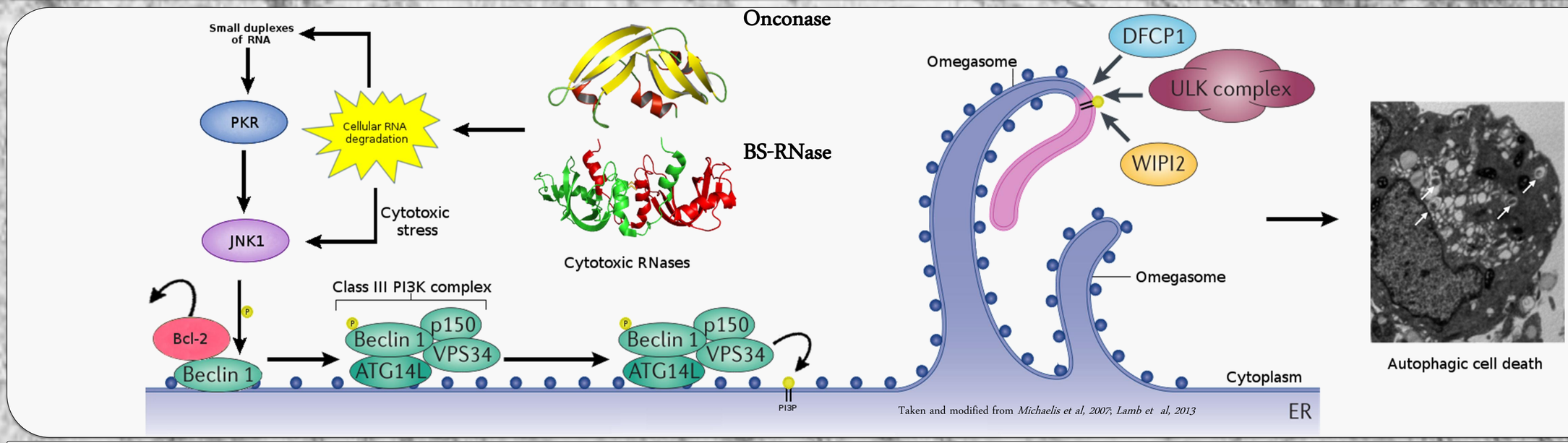
RNase L

- Antiviral non-secretory RNase linked to innate immunity.
- Autophagy induction by RNase L was reported in response to viral infections[5].

Objectives

- In basis of RNase L autophagy induction[5], to propose an hypothetical mechanism of induction of autophagic cell death in tumoral cells for vertebrate secreted cytotoxic RNases.
- To evaluate the future of cytotoxic ribonucleases as antitumoral agents.

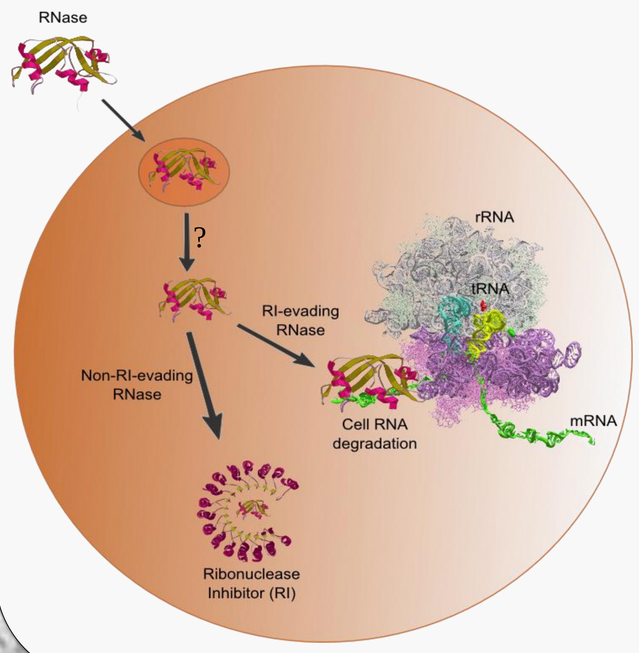
Induction mechanism of autophagic cell death by ribonucleases

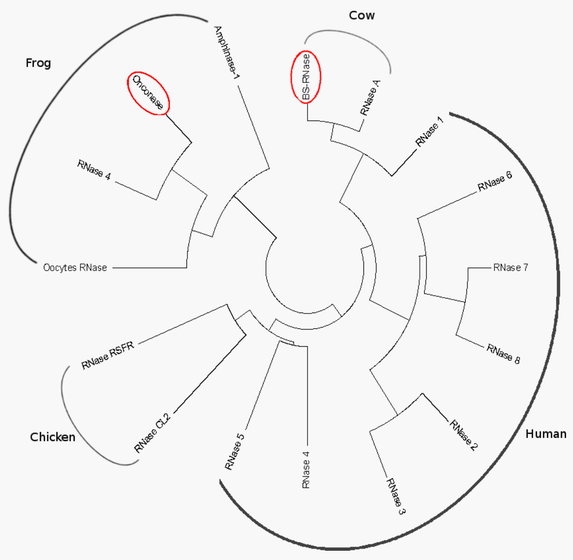


Abbreviations: Atg (Autophagy related); Bcl-2 (B-cell lymphoma 2); Beclin1 (BCL-2 interacting myosin/moesin-like coiled-coil protein 1); DFCP1 (double FYVE domain-containing protein 1); JNK (c-Jun N-terminal kinase); PKR (dsRNA-dependant protein kinase); ULK (UNK-51-like kinase); Vps (vacuolar protein sorting); WIPI (WD repeat domain phosphoinositide-interacting).

Structural determinants of the antitumoral activity of vertebrate secretory RNases

- Cationic properties.
- Ability of entering cells[7].
- Evasion of ribonuclease inhibitor. Specific for RNase A superfamily.
  - Suppression of RI-interacting residues (onconase).
  - Multimer formation (BS-RNase).





Philogenetic tree of selected members of RNAse A superfamily. Protein sequences were aligned by ClustalW and MEGA6 was used for the construction of the tree.

Reported antitumoral activities of cytotoxic RNases

	BS-RNase WT[2]	BS-RNase G38K[2]	Onconase[3]	Cell type
IC_{50}	~100 µg/ml	~18 µg/ml	~22 µg/ml	**Pancreatic cancer cells**
	> 200 µg/ml	~200 µg/ml	> 200 µg/ml	**Fibroblasts**
Cell growth inhibition at 50 µg/ml	~ 40 %	~75 %	~ 62.5%	**Pancreatic cancer cells**
	~ 17 %	~ 25 %	~ 30 %	**Fibroblasts**
Cell growth inhibition at 200 µg/ml	~ 60 %	~ 80 %	~ 65 %	**Pancreatic cancer cells**
	~ 40 %	~ 50 %	~ 35 %	**Fibroblasts**
Autophagosome formation at 200 µg/ml	13x	35x	4x	**Pancreatic cancer cells**
	3x	4x	1.5x	**Fibroblasts**

- Some phase III clinical trials were done with onconase in humans, showing an increase of median survival of the patients in comparison to the standard chemotherapeutic agent[8,9].

Conclusions

- Ribonuclease-dependant autophagic cell death in cancer cells is induced via Beclin-1, most probably by JNK1.
- Currently, BS-RNase G38K[2] is the best antitumoral RNase.
  - ⟶ High sequence identity with human ribonucleases
  - ⟶ Best reported activity in vitro
  - ⟶ Higher molecular weight than onconase
- More studies will be necessary to elucidate the exact mechanism and improve the antitumoral activity of cytotoxic ribonucleases.

Antitumoral activity improvement

- Enhancement of catalytic activity will result in higher antitumoral activity.
- Enhancement of cationic properties results in better selectivity to cancer cells.
- Higher molecular weight (multimers) would reduce the high renal accumulation and the rapid clearance from blood circulation found in clinical trials.
- Enhancement of the stability of BS-RNase dimer for RI avoidance.
- Ongoing studies (*Quintessence Biosciences*): Some mutants of HP-RNase (Evade™ Ribonucleases) are able to evade RI and target cancer cells more specifically.

References

1. **Denton, D., Xu, T. and Kumar, S.** (2014). Autophagy as a pro-death pathway. *Immunol. Cell Biol.* **93**, 35–42.
2. **Michaelis, M., Cinatl, J., Anand, P., Rothweiler, F., Kotchetkov, R., Deimling, A. Von, Doerr, H. W., Shogen, K. and Cinatl, J.** (2007). Onconase induces caspase-independent cell death in chemoresistant neuroblastoma cells. *Cancer Lett.* **250**, 107–116.
3. **Fiorini, C., Gotte, G., Donnarumma, F., Picone, D. and Donadelli, M.** (2014). Bovine seminal ribonuclease triggers Beclin1-mediated autophagic cell death in pancreatic cancer cells. *Biochim. Biophys. Acta* **1843**, 976–84.
4. **Fiorini, C., Cordani, M., Gotte, G., Picone, D. and Donadelli, M.** (2015). Onconase induces autophagy sensitizing pancreatic cancer cells to gemcitabine and activates Akt/mTOR pathway in a ROS-dependent manner. *BBA - Mol. Cell Res.* **1853**, 549–560.
5. **Siddiqui, M. A. and Malathi, K.** (2012). RNase L induces autophagy via c-Jun N-terminal kinase and double-stranded RNA-dependent protein kinase signaling pathways. *J. Biol. Chem.* **287**, 43651–64.
6. **Lamb, C. a, Yoshimori, T., and Tooze, S. a** (2013). The autophagosome: origins unknown, biogenesis complex. *Nat. Rev. Mol. Cell Biol.* **14**, 759–774.
7. **Chao, T. Y.; Raines, R. T.** (2011). Mechanism of Ribonuclease A Endocytosis: Analogies to Cell-Penetrating Peptides. *Biochemistry* **50**, 8374–8382.
8. **Mikulski SM, Chun H, Mittelman A, Panella T, Puccio C, Shogen K, Costanzi J** (1995) Relationship between response rate and median survival in patients with advanced non-small cell lung cancer: comparison of ONCONASE® with other anticancer agents. *Int J Oncol* **6**, 889–897.
9. **Mikulski SM, Costanzi JJ, Vogelzang NJ, McCachren S, Taub RN, Chun H, Mittelman A, Panella T, Puccio C, Fine R, Shogen K** (2002) Phase II trial of a single weekly intravenous dose of ranpirnase in patients with unresectable malignant mesothelioma. *J Clin Oncol* **20**, 274–281.