## Article

## Rapha Myr<sup>®</sup>, a Blend of Sulforaphane and Myrosinase, Exerts Antitumor and Anoikis-Sensitizing Effects on Human Astrocytoma Cells Modulating Sirtuins and DNA Methylation

Barbara Tomasello <sup>1,\*</sup>, Maria Domenica Di Mauro <sup>1</sup>, Giuseppe Antonio Malfa <sup>1</sup>, Rosaria Acquaviva <sup>1</sup>, Fulvia Sinatra <sup>2</sup>, Giorgia Spampinato <sup>3</sup>, Samuele Laudani <sup>2</sup>, Giusy Villaggio <sup>2</sup>, Anna Bielak-Zmijewska <sup>4</sup>, Wioleta Grabowska <sup>4</sup>, Ignazio Alberto Barbagallo <sup>1</sup>, Maria Teresa Liuzzo <sup>5</sup>, Elisabetta Sbisà <sup>6</sup>, Maria Grazia Forte <sup>7</sup>, Claudia Di Giacomo <sup>1</sup>, Massimo Bonucci <sup>8</sup> and Marcella Renis <sup>1,\*</sup>

- <sup>1</sup> Department of Drug Science, Section of Biochemistry, University of Catania, Viale A. Doria 6, 95125 Catania, Italy; mariadomenica.dimauro@virgilio.it (M.D.D.M.); g.malfa@unict.it (G.A.M.); racquavi@unict.it (R.A.); ignazio.barbagallo@unict.it (I.A.B.); cdigiac@unict.it (C.D.G.)
- <sup>2</sup> Department of Biomedical and Biotechnological Sciences, University of Catania, Via Santa Sofia 87, 95125 Catania, Italy; sinatra@unict.it (F.S.); s.laudani91@hotmail.it (S.L.); giusyvillaggio@gmail.com (G.V.)
- <sup>3</sup> Services Center B.R.I.T. of the University of Catania, 95124 Catania, Italy; giorgiaspampinato@unict.it
- <sup>4</sup> Nencki Institute of Experimental Biology, Polish Academy of Sciences, 3 Pasteur St, 02-093 Warsaw, Poland: a.bielak@nencki.gov.pl (A.B.-Z.); w.grabowska@nencki.gov.pl (W.G.)
- <sup>5</sup> Medical Clinic of Clinical Allergology and Immunology, 97018 Scicli, Italy; mariateresaliuzzo@gmail.com
- <sup>6</sup> Institute of Biomedical Technologies -National Research Council Bari, 70126 Bari, Italy; elisabetta.sbisa@ba.itb.cnr.it
- <sup>7</sup> Dept. Prevention ASL BA-SIAN Metropolitan Area Bari, 70120 Bari, Italy; mariagrazia.forte@asl.bari.it
- <sup>8</sup> Association Research Center for Integrative Oncology Treatments (ARTOI), 00165 Rome, Italy; maxbonucci@artoi.it
- \* Correspondence: btomase@unict.it (B.T.); renis@unict.it (M.R.); Tel.: +39-095-7384063 (B.T.); Fax: +39-095-7384220 (B.T.)



**Figure S1:** Cell morphology of SHSY5Y (neuroblastoma cells) untreated and treated for 24h with different concentrations of Rapha Myr<sup>®</sup>. Images were acquired by optical inverted light microscopy, original magnification 10x, scale bare 400µm.



**Figure S2:** Cell morphology of U87 (glioblastoma cells) untreated and treated for 24h with different concentrations of Rapha Myr<sup>®</sup>. Images were acquired by optical inverted light microscopy, original magnification 10x, scale bare 400µm.





**Figure S3:** Cell morphology of HFF1 (fibroblast cells) untreated and treated for 24h with different concentrations of Rapha Myr<sup>®</sup>. Images were acquired by optical inverted light microscopy, original magnification 1010x, scale bare 400µm.

## Figure S4



Figure S4: Abnormal mitotic spindles. The merge was made between spindle microtubules and DAPI-stained chromosomes. Scale bar: 20  $\mu m$ 

Figure S5 nuclei quantification



**Figure S5**: Nuclear morphology (DAPI) and percentages values of normal, small (s), misshappen or fragmented (m) nuclei in 1321N1 cells untreated and treated with of 0.5% and 1.25% v/v Rapha Myr<sup>®</sup> extract for 72 h.



**Figure S6:** Representative flow cytometry cell cycle histogram. Cell cycle analysis by flow cytometry G1, S, and G2/M phase in human astrocytoma (1321N1) cells treated with different concentrations of Rapha Myr<sup>®</sup> for 24h. For the graph see main manuscript (Figure 8A).





**Figure S7:** Representative flow cytometry scatter plots showing apoptosis of human astrocytoma (1321N1) cells stained with annexin V-fluorescein isothiocyanate (FITC)/propidium iodide following treatment with different concentrations of Rapha Myr<sup>®</sup> for 24h. For the graph see main manuscript (Figure 8B)