Proceedings of the LX SIGA Annual Congress
Catania, Italy – 13/16 September, 2016
ISBN **978-88-904570-6-7**

Poster Communication Abstract – 7.27

METABOLIC ENGINEERING OF YEAST FOR THE PRODUCTION OF PLANT AVENANTHRAMIDE ANALOGOUS ENDOWED WITH ANTIOXIDANT AND ANTIPROLIFERATIVE PROPERTIES

MOGLIA A.*, TRAPANI E.**, DI SCIPIO F.**, FINETTI F.***, YANG Z.**, SCHIAVO I.***, GOITRE L.**, BALDINI E.***, DONNINI S.***, TRABALZINI L.***, BERTA G.N.**, RETTA S.F.**

*) Department of Agricultural, Forest and Food Sciences, Università degli Studi di Torino, Largo Paolo Braccini 2, 10095 Grugliasco (Italy)
**) Department of Clinical and Biological Sciences, Università degli Studi di Torino, Regione Gonzole 10, 10043 Orbassano (Italy)
***) Department of Biotechnology, Chemistry and Pharmacy, Università degli Studi di Siena, Via Fiorentina 1, 53100 Siena (Italy)

plant secondary metabolites, avenanthramide, heterologous production, antioxidant

Biological synthesis of therapeutic drugs beneficial for human health using microbes offers an alternative production strategy. A novel fermentation system for the heterologous production of phenolic amides, N-(E)-*p*-coumaroyl-3-hydroxyanthranilic acid (Yeast avenanthramide I, Yav I) and caffeoyl-3-hydroxyanthranilic acid (Yav II), was set up by engineering a *S. cerevisiae* strain with two genes (4cl-2 from tobacco and hct from globe artichoke). These novel compounds exhibited a strong structural similarity with oat avenanthramides, a group of natural antioxidants. By developing a fermentation process, the production of yeast avenanthramides reached a final yield of 125 mg/L for YAv I and 22.5 mg/l for YAv II.

To examine the biological relevance of Yav we tested their anti-oxidant properties in MEF and the HeLa cell lines by analyzing their effects on master regulators of cell antioxidant responses, SOD2 and its transcriptional regulator FoxO1. Real Time PCR and Western Blot analysis suggested that yeast avenanthramides positively regulate the anti-oxidant defense mechanism through the up-regulation of FoxO1 and SOD2 expression level. Intriguingly, we demonstrated that cell treatment with YAv can revert molecular phenotypes caused by the loss of KRIT1, a protein involved in the human genetic disease Cerebral Cavernous Malformation.

In addition, we investigated the cell anti-proliferative potentiality of Yavs by using several colon, oral and lung cancer cell lines. As assessed by MTT assays, Yav I and Yav II significantly inhibited cell proliferation within 48-72 h of cell treatment. Furthermore, flow cytometry analysis revealed that cell treatment with Yav I and II arrested the cell cycle in G1 phase. The cell cycle arrest was associated with a significant decrease in Cyclin D1 expression both at transcriptional and translation levels. Epithelial-mesenchymal transition (EMT) is recognized as an important event in carcinoma progression. YAvs prevented EMT process in colon and oral cancer cell lines through the up-regulation of E-cadherin, which is the main protein of adherens junction that anchor epithelial cell to each other.