UNIVERSITY OF SALERNO

(DEPARTMENT OF CHEMISTRY AND BIOLOGY)

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(DEPARTMENT OF SCIENCE)

Ph.D. in Chemistry – XXXI Cycle (BIO/10 – Biochemistry)

Doctoral Thesis in

"The protein related to Pseudoxanthoma Elasticum regulates the purinergic system. New insight on the ABCC6 transporter"

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Abstract:

ABC (ATP-binding cassette) transporters are the largest superfamily of membrane proteins present in all organisms and they are particularly involved in transport of nutrients and drugs. Among these transporters we find ABCC6, belonging to sub-family C, which is an ATP-dependent transporter mainly present in the basolateral plasma membrane of hepatic and kidney cells. Mutations in the *ABCC6* gene are associated to the Pseudoxanthoma Elasticum (PXE), an autosomal recessive disease characterized by progressive ectopic mineralization processes at level of the skin, the retina and the vascular wall. It has been reported that PXE is caused in peripheral tissues by decreased levels of PP_i, a strong inhibitor of mineralization processes. In fact, the administration of PP_i reducing the effects of PXE is considered an efficient drug. It is known that the over-expression of ABCC6 in HEK293 cells results in the outflow of ATP and that is converted into AMP and PP_i by ENPP1 protein. Then, AMP is transformed into adenosine and P_i by CD73 protein; so ABCC6 protein could be involved both in providing extracellular adenosine and in the regulation of the purinergic system. Previous studies show that in *ABCC6*-silenced HepG2 cells there is a dysregulation of some genes involved in the mineralization processes. We performed experiments in order to evaluate the mechanism by which ABCC6 is able to promote this genic dysregulation. For this purpose, the ABCC6 transport activity was inhibited with an aspecific inhibitor of ABC transporters. The main results obtained in HepG2 cells are partially similar to those ones obtained in *ABCC6*-silenced HepG2 cells. The greatest effects were obtained on *NT5E* and on *ABCC6*. As an inhibitor, probenecid was used to reduce the transport activity of ABCC6 into HEK293 cells. In order to confirm that the observed effect is closely related to the inhibition of ATP transport, and not to the presence of probenecid, the HepG2 cells were also treated with adenosine and ATP. The results show that in the presence of 10 and 100 µM of adenosine or 50 and 500 µM of ATP, the effects of probenecid are reversed. In order to confirm the effects of probenecid on the transport activity in these cells was tested the quercetin, another ABC inhibitor; the data confirm those ones previously obtained with probenecid on the expression of *ABCC6* and *NT5E*. In HepG2 cells treated with doxorubicin, whose presence increases the expression of *ABCC6*, we observed a proportional increase of *NT5E*. Experiments with probenecid, adenosine and ATP were also performed in cells expressing *ABCC6* at different levels. The results show that in the presence of

probenecid in MDA-MB-231 cells there is no effect while in the HEK293 and HuH-7 cells there are poor effects. The effect of probenecid is obtained only in cells that over-express *ABCC6*; in all these cells, the addition of adenosine and ATP improves the expression of CD73. It was also analysed the cellular phenotype after the treatment with probenecid. Considering the role of CD73 in cellular migration processes, motility assays confirmed that HepG2 cells migrate more slowly, after this treatment.