MOLECULAR MECHANISM OF L-PROLINE INDUCED EPL-CELL FORMATION

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By

Ana Lonic, Bachelor of Science (Honours)

Department of Biochemistry

School of Molecular and Biomedical Science

University of Adelaide

Adelaide, South Australia

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TABLE OF CONTENTS

SUMMARY

During early embryogenesis pluripotent cells of the inner cell mass (ICM) give rise to a second pluripotent cell population known as the primitive ectoderm an obligate developmental intermediate and the substrate for gastrulation. The ICM and primitive ectoderm are distinguished on the basis of morphology, gene expression and differentiation potential. However, the signals and mechanisms involved in the transition form ICM to primitive ectoderm are not understood. Culture of ES cells in the presence of a conditioned medium MEDII leads to a transition of ES cells to a population of pluripotent primitive ectoderm-like (EPL) cells that are the *in vitro* equivalent of the primitive ectoderm. In terms of EPL cell formation the bioactive component of MEDII was identified as L-proline. In this thesis the molecular mechanism by which L-proline induces EPL-cell formation was elucidated.

As well as L-proline, short L-proline containing peptides were also shown to induce EPL-cell formation but different peptides displayed different abilities to induce the transition with some inducing the complete transition and others inducing morphology changes only. The mechanism of L-proline induced EPL-cell formation was shown to be independent of NK receptors. The mechanism of L-proline induced EPL-cell formation, as deduced from the results presented in this thesis, was suggested to involve the internalisation of L-proline via the SAT2 amino acid transporter into ES cells as competitive inhibitors of SAT2 prevented EPL-cell formation. MAPK signalling via the action of MEK1 was implicated in L-

proline induced EPL-cell formation as inhibitors of MEK1 prevented EPL-cell morphology, gene expression and differentiation potential in the presence of L-proline.

PI3K signalling was implicated in L-proline-induced EPL-cell morphology since PI3K inhibitor LY294002 maintained domed colonies in the presence of L-proline but failed to maintain an ES-cell gene expression profile and differentiation potential. Both MAPK and PI3K signalling were suggested to lie down-stream of L-proline action since treatment of ES cells with L-proline induced the activation of ERK1/2 and Akt down-stream effectors of MAPK and PI3K signalling respectively. A gene potentially involved in the PI3K-rnediated rnorphology change was *Lefty2*. Therefore, the mechanism of L-proline induced EPL-cell formation appears to involve internalisation of L-proline and at least two signalling pathways down-stream of L-proline, which regulate different components of the transition.