CHARACTERIZATION OF A549 LUNG HUMAN ALVEOLAR EPITHELIAL CELLS IN CULTURE

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Key words: A549, metabolism, alveolar cell culture

Introduction. The use of cell lines as models in medical research is a useful approach to perform experimentation of many and dissimilar aspects of cellular physiology and metabolism. Achievement of goals requires a quantitative characterization on the growth and metabolism of the cell line (kinetic performance) under experimental conditions. That characterization is in the majority of cases not performed.

A549 is a human epithelial alveolar type II (ATII) cell line widely employed for the *in vitro* study of many aspects in respiratory research.[1-3] There is no quantitative evidence in respect to the growth and metabolism of those cells growing in RPMI-1640 culture medium supplemented with Fetal Bovine Serum (FBS). In the present work the kinetic performance of both low and high passage number A549 cells growing in RPMI-1640 plus 10% FBS was determined. Two nutritional conditions were evaluated: 2 mM Gln/11 mM Glc and 4 mM Gln/22 mM Glc. Kinetic profiles on cell growth, metabolite consumption and production as well as the main kinetic and stoichiometric parameters of growth and metabolism were determined.

Methods. Cell line: A549, ATCC CCL-185, low and high passage number. Culture medium: RPMI-1640 Cat. R4130 supplemented with 2.0 g/L $NaHCO_3$, 2 mM glutamine and 11 mM glucose when required (all from Sigma, St. Luis, MO). Cultures were performed in triplicate in 6 well plates containing 1.5 mL culture medium per well using a CO_2 incubator at 37°C and humidity saturation. Cultures were seeded at Cell concentration and viability were measured by trypan blue dye exclusion and counted in a Neubauer chamber at 10X objective magnification. Metabolite concentration glucose, lactate and glutamine were measured enzimatically in an YSI-2700 analyzer (Yellow Sprigs, OH).

Results. Cell growth and metabolite consumption kinetics under identical culture conditions revealed that A549 cells at high passage numbers suffered from dramatic alterations in their physiology and metabolism in comparison with cells at low passage number (see Fig. 1). Light microscopy observations also showed radical morphology changes. The characteristic epithelial cuboidal shape observed at low passage number turn in to an epithelial fibroid shape at high passage number (see Fig. 2).

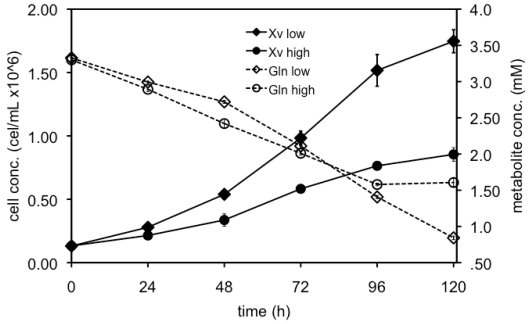

Fig.1 Growth and metabolite consumption profile in A549 alveolar cell line. Comparison between cells at low and high passage number. Xv, viable cell concentration. Gln, glutamine.

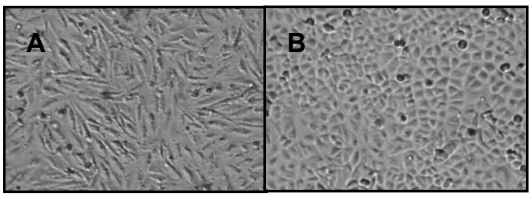

Fig. 2 . Light microscopy of confluent A549 alveolar cell line. 40X magnification. Morphological comparison between high (A), and low (B) passage number.

Conclusions. The number of cell divisions (passage number) and cell culture conditions drastically impact metabolism and morphology of A549 alveolar epithelial cell line.

Acknowledgements. Financial Support from CONACyT CB2010-155653 and technical assistance by Rosita Nieto.

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