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UNIVERSITA' DEGLI STUDI DI PISA
Facoltà di Scienze Matematiche, Fisiche e Naturali
Corso di Laurea Specialistica in Scienze e Tecnologie Biomolecolari
Curriculum Molecolare Cellulare

TESI DI LAUREA SPECIALISTICA

Effects of Methyl Cycle Substrate Availability on Epigenetic Stability of Human Embryonic Stem Cells

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ABSTRACT

A link has been hypothesised to occur between suboptimal maternal nutrition and impaired foetal development leading to a predisposition to a range of adult pathologies. As a clear connection between dietary intake of methyl group donors and epigenetic defects has been demonstrated both *in vivo* and *in vitro*, this project had the purpose of generating a disruption into the methyl/folate cycle to investigate DNA methylation alterations during human preimplantation embryo development, using human embryonic stem cells (hESCs) as an *in vitro* model. In particular, HUES-7 stem cells were employed and cultured using either standard or methyl deficient media to test this hypothesis. After the treatments, that included an inhibitor of a key enzyme of the cycle, Differentially Methylated Regions (DMRs) of six imprinted genes were analysed and assessed for their methylation status at Cytosine-phospho-Guanosine (CpG) sites. As a consistent decrease of methylation was observed for the gene *H19* in treated cultures, its allelic expression was then investigated and an initial process of Loss Of Imprinting (LOI) was found. Additionally, global DNA MethylTransferase (DNMT) activity was examined and a statistically significant decrease in treated samples was detected. Finally, hESCs were differentiated into Embryoid Bodies (hEBs), which were compared and stained for pluripotency and germ-layer specific markers. Consistently different expression of *OCT-4* and *NANOG* was noticed for treated-culture derived hEBs.

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RIASSUNTO

Negli ultimi anni è stata ipotizzata l'esistenza di un rapporto tra livelli sub-optimali di composti donatori di metile nell'alimentazione materna e un aberrante sviluppo embrionale, spesso causa di predisposizione a varie patologie nell'età adulta. Poiché è stata dimostrata sia *in vitro* che *in vivo* una chiara relazione tra apporto, con la dieta, di molecole donatrici di gruppi metile e alterazioni epigenetiche, questo progetto ha avuto lo scopo di generare e indurre scompensi nei cicli congiunti del metile e del folato per studiare alterazioni nel dinamico processo di metilazione del DNA che avviene durante lo sviluppo embrionale pre-impianto, con l'utilizzo come modello di cellule staminali embrionali umane (hESCs). In particolare, si è fatto uso di una linea cellulare, HUES-7, coltivata usando sia terreni standard sia terreni a bassa concentrazione di donatori di gruppi metile. Successivamente ai trattamenti, che hanno incluso anche un inibitore di uno degli enzimi chiave del ciclo del metile, specifiche Differentially Methylated Regions (DMRs) di sei geni imprinted sono state analizzate annotandone lo stato di metilazione dei siti Citosina-fosfo-Guanina (CpG) che le compongono. Poiché un rilevante fenomeno di demetilazione è stato osservato per il gene *H19*, ne è stata esaminata l'espressione allelica portando alla luce un processo iniziale di perdita di imprinting (LOI). Inoltre, è stata misurata l'attività globale degli enzimi DNA MetilTransferasi (DNMTs) e un decremento statisticamente significativo è stato individuato nei campioni trattati. Infine, le cellule sono state indotte al differenziamento in corpi embrioidi (hEBs) che sono stati successivamente comparati tra loro e saggiati per marcatori di pluripotenza e foglietto germinativo specifici tramite tecniche immunoistochimiche. Un diverso livello di espressione è

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stato individuato per *OCT-4* e *NANOG* negli hEBs derivati da colture trattate con terreni a bassa concentrazione di donatori di gruppi metile.

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ABBREVIATION LIST

3-DZA = 3-Deazaadenosine.
ANOVA = Analysis Of Variance.
ART = Assisted Reproductive Technology.
bFGF = basic Fibroblast Growth Factor.
BGK = BresaGen Knockout serum replacement.
BMP = Bone Morphogenetic Protein.
BWS = Beckwith-Wiedemann Syndrome.
cDNA = complementary DNA.
CM = Conditioned Medium.
CpG = Cytosine-phospho-Guanine.
CTCF = CCCTC binding Factor.
CTL = Cytotoxic T Cell.
Cys = Cysteine.
d = day.
D-MEM = Dulbecco's Modified Eagle Medium.
DMR = Differentially Methylated Region.
DMSO = DimethylSulfOxide.
Dnmt/DNMT = DNA MethylTransferase.
dNTP = deoxyriboNucleotide TriPhosphate.
DOHaD = Developmental Origin of Health and Disease.
DTT = DiThioThreitol.
EB = Embryoid Body.
EDTA = EtylenDiamyneTetraAcetic acid.
EGF = Epidermal Growth Factor.
EHS = Engelbreth-Holm-Swarm.
ELISA = Enzyme Linked ImmunoSorbent Assay.
ESC = Embryonic Stem Cell.
EtOH = Ethanol.
FA = Folic Acid.
FBS = Foetal Bovine Serum.
-FGF = high FGF cultures, supplemented with 100 ng/ml bFGF.
gDNA = genomic DNA.
GH = Growth Hormone.
Gln = Glutamine.
Gly = Glycine.
GRB = Growth factor Receptor-Bound protein.
GZMB = Granzyme B.
GαH = Goat α-Human.
h = human.
HRP = HorseRadish Peroxydase.
IAP = Intracistronic A Particle.
ICF = Immunodeficiency-Centromeric Instability-Facial Anomalies
ICM = Inner Cell Mass.
ICR = Imprinting Control Region.
IGF = Insulin-like Growth Factor.
IGFR = Insuline-like Growth Factor Receptor.
IsPrOH = Isopropanol.

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IVF = *In Vitro* Fertilisation.
KCNQ = Potassium Channel, voltage-gated, KQT-like subfamily.
KO-DMEM = Knock-Out Dulbecco's Modified Eagle Medium.
KOH = Knock-Out Dulbecco's modified eagle medium Hyclone serum.
KSR = Knockout Serum Replacement.
LIF = Leukaemia Inhibitory Factor.
LOH = Loss Of Heterozygosity.
LOI = Loss Of Imprinting.
m = mouse.
M6P = Mannose-6-Phosphate.
MAT = Methionine Adenosyl Transferase.
MEF = Mouse Embryonic Fibroblast.
MESP = Mesodermal Posterior 1
Met = Methionine.
Me-THR = Methyl-TetraHydroFolate.
MG = Methyl Group.
$MgCl_2$ = Magnesium chloride.
MMC = MitoMycin C.
MMR = MisMatch Repair.
MS = Methionine Synthase.
MSLH = Methyl Substrate Low Hyclone serum.
MαH = Mouse α-Human.
$NaHSO_3$ = Sodium Bisulfite.
NaOAc = Sodium Acetate.
NaOH = Sodium Hydroxide.
NDNL = Necdin-Like.
NEAA = Non Essential Amino Acids.
NFkB = Nuclear Factor kappa-B.
NGF =Nerve Growth Factor.
Oct/OCT = Octamer binding Transcription factor.
p = passage.
PBS = Phosphate Buffered Saline.
PcG = Polycomb Group protein.
PCI = Phenol Cloroform Isoamyl alcohol.
PCR = Polymerase Chain Reaction.
PDGF = Platelet-Derived Growth Factor.
PEG = Paternally Expressed Gene.
Pen/Strep = Penicillin/Streptomicin.
PFA = para-Formaldehyde.
PLG = Phase Lock Gel.
PWS = Prader-Willi Syndrome.
RaαM = Rabbit α-Mouse.
RFLP = Restriction Fragment Length Polymorphism.
RT = Room Temperature.
SAH = S-Adenosyl Homocysteine.
SAHh = S-Adenosyl Homocysteine hydrolase.
SAM = S-Adenosyl Methionine.
Ser = Serine.
SMAD = Sma/Mothers-Against-Decapenthaplegic homologue.
SNP = Single Nucleotide Polymorphism.
SNRPN = Small Nuclear Ribonucleoprotein Polypetide N.

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Sox/SOX = Sex determining region of the Y chromosome-related box containing gene.
SSEA = Stage-Specific Embryonic Antigen.
Stat/STAT = Signal Transducer and Activator of Transcription.
T = Brachyury.
TBX = T Box.
TGF = Transforming Growth Factor.
THF = TetraHydroFolate.
THFR = TetraHydroFolate Reductase.
Thr = Threonine.
TNF = Tumor Necrosis Factor.
Usp/USP = Ubiquitin-specific processing protease.
Vit = vitamin.
Wsb/Nf1 = WD repeat and socs box-containing protein /Neurofibromin.
Zim/ZIM = Zinc finger protein imprinted gene.
Znf/ZNF = Zinc Finger protein.
β-ME = β-Mercapthoethanol.

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