Ministero dell'Università e della Ricerca

Direzione generale della ricerca

RENDICONTO DI SPESA
CINQUE PER MILLE

ENTI DELLA RICERCA SCIENTIFICA E DELL'UNIVERSITÀ

ANNO FINANZIARIO

___2021____[1]

[1] Indicare l'anno finanziario al quale si riferisce l'erogazione.

ENTE BENEFICIARIO

Denominazione Ente	**FONDAZIONE SANTA LUCIA IRCCS**
Codice fiscale	97138260589
Sede legale	Via Ardeatina, 306 – 00179 Roma
Indirizzo PEC	fondazionesantalucia@pec.fondazionesantalucia.it
Scopo dell'attività sociale	**Miglioramento della diagnosi e cura delle patologie neuroinfiammatorie e neurodegenerative con particolare attenzione alla neuroriabilitazione di alta specialità.**
Nominativo legale rappresentante	**MARIA ADRIANA AMADIO**

CONTRIBUTO PERCEPITO

Data erogazione	**11/10/2022**
Importo	**€ 40.451,58**

SPESE SOSTENUTE [2]

VOCI DI SPESA	COSTO COMPLESSIVO	QUOTA FINANZIATA CINQUE PER MILLE
DI FUNZIONAMENTO		
Risorse umane *Dettaglio spese:* 1. Prestazione di Lavoro Coordinato e Continuativo_Biologo Ricercatore dal 02/11/2022 al 31/05/2023.	21.590,01	21.590,01
Acquisto beni e servizi *Dettaglio spese:* 1. Prodotti chimici e reagenti	14.816,48	14.816,48
ALTRE VOCI DI SPESA [3]		
Dettaglio spese: 1. Spese generali	4.045,00	4.045,00
ACCANTONAMENTI PROGETTI PLURIENNALI [4]		
Dettaglio spese: 1. …		
TOTALE	40.451,58	40.451,58

Il seguente rendiconto è pubblicato al seguente indirizzo web
https://www.hsantalucia.it/fondazione/amministrazione-trasparente

Data 21/11/2023

Il Legale Rappresentante
(*Firma digitale*)

Si autorizza al trattamento dei dati ai sensi del d.lgs.196/2003 e al Regolamento (UE) 2016/679 (GDPR)

[2] Evidenziare la loro riconduzione alle finalità ed agli scopi istituzionali del soggetto beneficiario.
[3] Altre voci di spesa comunque destinate ad attività direttamente riconducibili alle finalità e agli scopi istituzionali del soggetto beneficiario.
[4] Eventuali accantonamenti delle somme percepite per la realizzazione di progetti pluriennali, con durata massima triennale, fermo restando l'obbligo di rendicontazione successive al loro utilizzo.

SANTA LUCIA

Scientific Report for the project
" Innovative technology for the study of neurodegenerative deseases".
Finanziamento 5XMILLE ANNO 2021

Currently, there are ethical and technical limitations for the apply of adult and embryonic human stem cells in research and regenerative medicine For this reason, a huge amount of effort has gone into developing functional equivalents of human Embryonic Stem Cells that do not involve the destruction of human embryos or eggs; in particular, research has focused on the identification of the pluripotency-inducing factors that could take somatic cells back to an undifferentiated condition. Recently Yamanaka and his collaborators demonstrated that the over-expression of a combination of just four transcription factors, octamer 3/4 (Oct3/4), SRY box– containing gene 2 (Sox2), Kruppel-like factor 4 (Klf4), and c-Myc, was sufficient to reprogram murine fibroblasts to an embryonic-like state .These reprogrammed cells were called induced pluripotent stem cells (iPSCs) and within months, the same group succeeded in generating iPSCs by introducing the human orthologs of the four transcription factor–encoding genes into human fibroblasts (Takahashi, et al., 2007). iPSCs can also be generated from patients and today are widely applied as models for the study of human diseases mechanisms and therapies of human diseases. This is particularly important for diseases that lack adequate *in vitro* or animal models, especially for disorders affecting the brain and the heart. These disease/mutation-specific cell lines offer an opportunity to map out the developmental course of complex medical conditions, in a manner not possible through animal research alone or by observing patients.
In the light of such technical improvement in generating cells that potentially can give rise at almost all mature human cells/tissue, I started to work with human samples obtained in collaboration with Policlinico Tor Vergata. In particular fibroblasts obtained from patients affected by progressive supranuclear palsy and by SLA have been reprogrammed to induced pluripotent stem cells using episomal vectors according to protocols already described in the literature. In short, 300,000 cells per reaction have been electroporated using the Nucleofector instrument already present in our laboratories, with 3µg of a mix of three episomal vectors based on the pCXLE plasmid expressing OCT4, KLF4, SOX2, L-MYC, LIN-28, sh-p53 using the standard protocol of nucleofection given by the manufacturer. The cells have been plated until the following day in culture medium for fibroblasts without the addition of antibiotics and then transferred under normal culture conditions. On the sixth day after nucleofection, fibroblasts have been detached and counted and 140,000 cells have been plated on a 100 mm plate previously coated with matrigel. The following day, the fibroblast culture medium have been replaced with a specific colture medium for stem cells, with the addition of antibiotics. After 25-40 days from nucleofection, the first colonies of iPS cells have been manually pick up, fragmented and seeded individually (step 1, p1) on a 24-well plate covered with Matrigel. For each reprogramming experiment, at least 3-5 iPSC clones, selected by morphology, have been expanded from p1. From p2 iPSC lines will be maintained in 6-well plates and weekly, peeled off with 0.5 mM pH 8 EDTA and expanded The obtained iPS cell lines have been evaluated for the expression of stem cell markers through immunofluorescence and Real Time PCR analyses. For immunofluorescence analysis, cells have been fixed in 4% paraformaldehyde for 20 minutes at room temperature and then permeabilized and blocked with saline (PBS), 10% goat serum (NGS), 1% bovine serum albumin (BSA), 0.1% Triton X-100 for 45 minutes at room temperature; then cells have been incubated until the next day at 4 ° C with specific primary antibodies ,anti-Oct4, anti-Tra-1-60 , and anti SSEA4 diluted in PBS 5% BSA. The following morning, the cells have been incubated for 1h at room temperature with the appropriate secondary antibodies and the nuclei labelled with Hoechst 33342 .Real Time PCR to verify the expression of stem cell markers have been conducted starting from total RNA extracted from iPS cells using an extraction kit according to the manufacturer's instructions. The RNA thus obtained have been back-transcribed into cDNA using the PrimeScript RT Reagent Kit and analyzed for the expression of the various genes of interest through the use of specific primers and the SYBR Green assay. JS iPSCs were differentiated

in vitro through embryoid bodies formation to analize the expression of three germ layers markers, through the following protocol: iPSCs from an entire 6-well dish were chemically detached and then plated in a petri dish in floating condition. Stemflex medium was then gradually switched to KOSR medium, in 3 days and cells mantained in culture for 14 days. Thus the RNA was back-transcribed and the analyzed by Real time PCR for at least two markers for each embryonic germ layer.

References

Kazutoshi Takahashi 1, Koji Tanabe, Mari Ohnuki, Megumi Narita, Tomoko Ichisaka, Kiichiro Tomoda, Shinya Yamanaka. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007 Nov 30;131(5):861-72. doi: 10.1016/j.cell.2007.11.019.