The International Journal of Biochemistry & Cell Biology 41 (2009) 1229–1232

Contents lists available at ScienceDirect

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

Medicine in focus

Treacher Collins syndrome: Unmasking the role of *Tcof1*/treacle

Daisuke Sakai[a], Paul A. Trainor[a,b,*]

[a] *Stowers Institute for Medical Research, 1000 East 50th Street, Kansas City, MO 64110, USA*
[b] *University of Kansas School of Medicine, 3901 Rainbow Boulevard, Kansas City, KS 66160, USA*

ARTICLE INFO

Article history:
Received 18 August 2008
Received in revised form 28 October 2008
Accepted 29 October 2008
Available online 5 November 2008

Keywords:
Treacher Collins syndrome
Tcof1/treacle
P53
Neural crest cells
Ribosome biogenesis

ABSTRACT

Treacher Collins syndrome (TCS) is a rare congenital birth disorder characterized by severe craniofacial defects. The syndrome is associated with mutations in the *TCOF1* gene which encodes a putative nucleolar phosphoprotein known as treacle. An animal model of the severe form of TCS, generated through mutation of the mouse homologue *Tcof1* has recently revealed significant insights into the etiology and pathogenesis of TCS (Dixon and Dixon, 2004; Dixon et al., 2006; Jones et al 2008). During early embryogenesis in a TCS individual, an excessive degree of neuroepithelial apoptosis diminishes the generation of neural crest cells. Neural crest cells are a migratory stem and progenitor cell population that generates most of the tissues of the head including much of the bone, cartilage and connective tissue. It has been hypothesized that mutations in *Tcof1* disrupt ribosome biogenesis to a degree that is insufficient to meet the proliferative needs of the neuroepithelium and neural crest cells. This causes nucleolar stress activation of the p53-dependent apoptotic pathway which induces neuroepithelial cell death. Interestingly however, chemical and genetic inhibition of p53 activity can block the wave of apoptosis and prevent craniofacial anomalies in *Tcof1* mutant mice [Jones NC, Lynn ML, Gaudenz K, Sakai D, Aoto K, Rey JP, et al. Prevention of the neurocristopathy Treacher Collins syndrome through inhibition of p53 function. Nat Med 2008;14:125–33]. These findings shed new light on potential therapeutic avenues for the prevention of not only TCS but also other congenital craniofacial disorders which share a similar etiology and pathogenesis.

1. Introduction

Craniofacial abnormalities are a recognized component of approximately one-third of all congenital birth defects (Gorlin et al., 1990). Treacher Collins syndrome (TCS, OMIM number 154500) is one of the most severe congenital disorders of craniofacial development and is extremely rare, occurring with an incidence of 1 in 50,000 live births. TCS was first described by Treacher Collins in 1900 after observation of two individuals with similar facial abnormalities (Treacher Collins, 1900). Subsequently in 1940, Franceschetti and Klein (1949) described the condition based on their own observations as mandibulofacial dysostosis. Characteristic abnormalities associated with TCS/mandibulofacial dysostosis include cleft palate, hypoplasia of the facial bones, particularly the mandible and zygomatic complex, downward slanting of the palpebral fissures with colobomas of the lower eyelids and deformity of the external ear. Other clinical features of TCS may include conductive hearing loss (Phelps et al., 1981) along with defects in brain development such as microcephaly and mental retardation. The gene responsible for TCS was identified on chromosome 5 and named *TCOF1* (Treacher Collins Syndrome Collaborative Group, 1996). The identification of all 26 exons of *TCOF1* has unveiled more than 100 family specific mutations which include splicing mutations, insertions, missense and nonsense mutations as well as deletions. TCS is an autosomal dominant disorder and molecular analysis of the *TCOF1* gene has determined that 40% of infants born with TCS have inherited one mutated copy of *TCOF1* while 60% arise as the result of *de novo* mutations (Splendore et al., 2003). The majority of mutations result in truncated proteins suggesting the importance of the C-terminal domain for Treacle function. Although there are inter- and intrafamilial variations ranging from mild to severe, there is no genotype/phenotype correlation.

2. Pathogenesis

Neural crest cells are a multipotent, stem and progenitor cell population, formed in the neural ectoderm at the boundary with non-neural ectoderm along the entire body axis during early embryogenesis. Neural crest cells undergo an epithelial–mesenchymal transition and in the cranial region, these cells delaminate from the neural ectoderm and concomitant with their expression of *Sox10* (Fig. 1), they migrate over extensive distances to the periphery of the face giving rise to most of the cartilage, bone,

* Corresponding author at: Stowers Institute for Medical Research, 1000 East 50th Street, Kansas City, MO 64110, USA. Tel.: +1 816 926 4414.
E-mail address: pat@stowers-institute.org (P.A. Trainor).

1357-2725/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biocel.2008.10.026

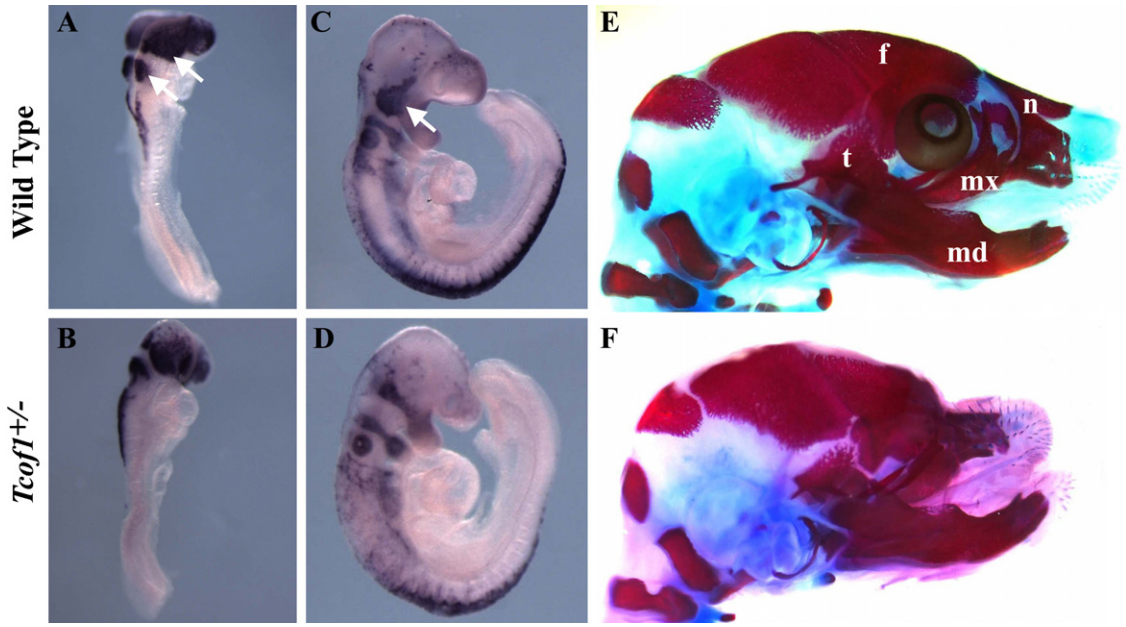

Fig. 1. Labelling of e8.5 and e9.5 wild type (A and C) and *Tcof1*$^{+/-}$ (B and D) mouse embryos via *in situ* hybridization with a *Sox10* riboprobe which is commonly used to mark migrating neural crest cells. Arrows highlight the decrease in the number of neural crest cells in TCS affected embryos. Alizarin red and alcian blue staining of bone and cartilage, respectively, in e18.5 wild type (E) and *Tcof1*$^{+/-}$ (F) embryos demonstrating the cranioskeletal hypoplasia characteristic of Treacher Collins syndrome that manifests from the earlier neural crest cell deficit. f, frontal; md, mandibular; mx, maxillary; n, nasal; and t, temporal bones (unpublished photos from the Trainor lab).

connective and peripheral nervous tissues in the head (Noden, 1983; Couly et al., 1998; Chai et al., 2000). Most disorders of craniofacial development are thought to be caused by defects in the formation, proliferation, migration, and/or differentiation of cranial neural crest cells. TCS is no exception as almost all of the cranioskeletal anomalies characteristically observed in TCS individuals involve tissues which are derived from neural crest cells. Hence abnormal neural crest migration, ectopic cell death and inappropriate differentiation have all been hypothesized as underlying causes of TCS (Wiley et al., 1983; Sulik et al., 1987; Poswillo, 1988; Sulik et al., 1988). However, until recently there was little experimental cellular or biochemical evidence to support any of these mechanistic hypotheses.

To precisely understand the pathogenetic mechanism of TCS, it is essential to elucidate the cellular and biochemical function of Treacle, the protein encoded by *TCOF1*. Treacle is a relatively simple 144 kDa protein that consists of at least three distinct domains, including unique amino and carboxy termini and a characteristic central repeat domain (Dixon et al., 1997; Wise et al., 1997). Putative nuclear export and import signals are seen at the N-terminus and C-terminus, respectively. It has been reported that the C-terminal domain is important for nucleolar localization of treacle (Marsh et al., 1998), and that perhaps the intracellular localization of treacle is very dynamic. Within the central domain, treacle contains multiple casein kinase II and protein kinase C phosphorylation site repeats. This is consistent with the fact that treacle is highly phosphorylated and associates with casein kinase II *in vitro* (Isaac et al., 2000). However, to date it has not been determined if phosphorylation is required for normal treacle function nor if it plays an important role in its subcellular localization.

Immunofluorescence studies have revealed that treacle colocalizes with upstream binding factor (UBF) and RNA polymerase I in the nucleolus (Valdez et al., 2004). Furthermore, biochemical analyses of treacle via *in vitro* siRNA-mediated knockdown demonstrated that treacle is essential for the proper transcription of rDNA, which is consistent with its structural homology to Nopp140, another nucleolar protein which also regulates rDNA transcription (Chen et al., 1999). Treacle has also been identified as a constituent of human Nop56-associated pre-ribosomal ribonucleoprotein (pre-rRNPs) complexes (Hayano et al., 2003) that 2′-*O*-methylate pre-ribosomal RNA during the early stages of pre-RNA processing in the nucleolus (Valdez et al., 2004). These data imply that treacle is contained within an RNP complex in the nucleolus and may be specifically involved in governing the ribosome biogenesis process.

Recently an essential role for treacle, in ribosome biogenesis was demonstrated *in vivo* (Dixon et al., 2006). Mice haploinsufficient for *Tcof1* exhibited diminished mature ribosome production as measured by the levels of 28S rRNA. This deficiency was observed in the neural ectoderm as well as in the neural crest but not in tissues such as the endoderm implying that treacle may function as a tissue-specific regulator of ribosome biogenesis (Dixon et al., 2006). The deficiency in ribosome biogenesis correlated with decreased proliferation in both the neural ectoderm and neural crest cells observed in *Tcof1* mutants. Consequently it has been hypothesized that deficient ribosome biogenesis is insufficient to meet the cellular and metabolic needs of these highly proliferative cell populations during embryogenesis and more specifically it is directly responsible for the high levels of cell death observed in the neural ectoderm at the time of neural crest formation (Dixon et al., 2006). Since the neural ectoderm cells are the precursors of the neural crest it is not surprising that the generation of neural crest cells is impaired. Furthermore, this can account for the characteristic hypoplasia of cranioskeletal elements observed in TCS individuals (Fig. 1).

The majority of mutations identified in humans are predicted to result in 3′ truncations of treacle and loss of the nuclear import signals. This firmly implies that the nuclear and nucleolar subcellular localization of treacle is critical to its ribosome biogenesis functions and moreover that ribosome biogenesis and consequently neural crest cell formation are similarly impaired in TCS patients.

3. Therapy

Infants with moderate or severe hypoplasia of the mandible and zygomatic complex typically have cleft palate along with narrowed airways and consequently experience major breathing and feeding

problems. In severe cases many newborn infants will require an immediate tracheostomy. At defined ages or when specific developmental milestones have been reached, extensive plastic surgery can help to restore the structure of the face. Bone grafts can be performed to build up the underdeveloped facial bones such as those surrounding the eyes. The ears can also be reconstructed and together with implantation of conductive auditory aids, hearing can be restored. Mandibular distraction can be performed to lengthen the jaw which can alleviate critical breathing and feeding problems and may also improve appearance as well. It is important to stress that the care of individuals affected by TCS requires a multidisciplinary approach and may involve intervention from a number of healthcare professionals both pre- and post-operatively. Excellent outcomes are achievable through a comprehensive, well-coordinated and integrated treatment plans incorporating craniofacial surgeons, orthodontists, ophthalmologists, otolaryngologists and speech pathologists. However, the results are often variable and may not be fully corrective, hence considerable effort should also be invested into examining therapeutic avenues of prevention.

In this regard, a recent study has not only deepened our understanding of the etiology and pathogenesis of TCS but more importantly has shed new light on potential avenues for the therapeutic prevention of TCS (Jones et al., 2008). Using a mouse model of TCS, it was discovered that p53 protein was activated and stabilized in the neuroepithelium of mutant embryos, and furthermore, that numerous p53-responsive pro-apoptotic genes, such as *Ccng1*, *Trp53inp1*, *Pmaip1*, *Perp* and *Wig1*, were also ectopically expressed in the neuroepithelium correlating with the high levels of neuroepithelium-specific cell death observed in *Tcof1* mutants. Previously, it has been well established that p53 is a tumor suppressor gene that functions in many critical events such as cell cycle regulation, DNA damage, centrosome amplification and apoptosis (Levine, 1997). Interestingly, nucleolar stress can also induce the activation of a p53-dependent checkpoint and subsequent cell cycle arrest and apoptosis (Rubbi and Milner, 2003). This suggests that haploinsufficiency of treacle which results in deficient ribosome biogenesis probably causes nucleolar stress activation and stabilization of p53. This in turn leads to the activation of numerous pro-apototic genes which can account for the high degree of neuroepithelial-specific apoptosis observed in *Tcof1* mutants.

This finding raises the possibility that inhibition of p53 activity may be able to block neuroepithelial apoptosis, restore the neural crest cell population and prevent the craniofacial anomalies characteristic of TCS. Indeed, in *Tcof1*$^{+/-}$ embryos treated in utero with pifithrin-α (a specific inhibitor of p53 activity), a dose- and exposure-dependent inhibition of neuroepithelial apoptosis and rescue of cranioskeletal development was observed (Jones et al., 2008). A similar but more efficient rescue was also observed when p53 activity was blocked genetically. Removal of one or two copies of p53 from the *Tcof1*$^{+/-}$ background revealed a dose-dependent inhibition of neuroepithelial apoptosis and prevention of cranioskeletal anomalies characteristic of TCS. This study by Jones et al. (2008) possibly represents the first successful rescue of a congenital neurocristopathy and provides an attractive model for the prevention of other craniofacial birth defects of similar etiology and pathogenesis involving neuroepithelial apoptosis.

However, a major caveat to inhibition of p53 as a therapeutic avenue for prevention of craniofacial anomalies is that p53 performs a number of important cellular functions not least of which is its role as a tumor suppressor. The loss of p53 frequently results in tumor formation and in fact 75% of *p53*$^{-/-}$ mice develop tumors within 3–6 months (Donehower et al., 1992). Therefore, it is essential to interrogate the downstream effectors of p53-dependent apoptosis, specifically the genes without any link to cancer and tumor progression as potential minimal risk candidates for the therapeutic prevention of TCS and other craniofacial syndromes.

Despite rescuing the craniofacial defects through inhibition of p53, ribosome biogenesis was still surprisingly impaired in *Tcof1*$^{+/-}$ mutants. This implies firstly that deficient ribosome biogenesis alone is not sufficient to generate the Treacher Collins syndrome phenotype. Secondly, deficient ribosome biogenesis may not be the trigger responsible for activation of the p53 and subsequent neuroepithelial apoptosis. Thirdly, there may be other non-ribosomal or non-nuclear functions for treacle during embryogenesis. In this regard, it was recently determined by *in silico* analysis that the N-terminus of Treacle contains a LisH motif which shares homology with the N-terminal region of Lis1 (Emes and Ponting, 2001). This motif contributes to microtubule binding, dimerization, protein half-life and localization (Gerlitz et al., 2005). This suggests that in addition to any nuclear import and export signals, the LisH motif may also be important for the dynamic localization of treacle. Furthermore, it may also be critical for as yet undiscovered functions of treacle which must be fully explored in order to fully appreciate the etiology and pathogenesis of TCS. The potential outcomes of course include providing a better understanding of the roles of *Tcof1* during embryogenesis and also possibly identifying other therapeutic avenues for the prevention and repair of TCS and other craniofacial malformation syndromes.

References

Chai Y, Jiang X, Ito Y, Bringas Jr P, Han J, Rowitch DH, et al. Fate of the mammalian cranial neural crest during tooth and mandibular morphogenesis. Development 2000;127:1671–9.

Chen HK, Pai CY, Huang JY, Yeh NH. Human Nopp140, which interacts with RNA polymerase I: implications for rRNA gene transcription and nucleolar structural organization. Mol Cell Biol 1999;19:8536–46.

Couly G, Grapin-Botton A, Coltey P, Ruhin B, Le Douarin NM. Determination of the identity of the derivatives of the cephalic neural crest: incompatibility between *Hox* gene expression and lower jaw development. Development 1998;125:3445–59.

Dixon J, Dixon MJ. Genetic background has a major effect on the penetrance and severity of craniofacial defects in mice heterozygous for the gene encoding the nucleolar protein Treacle. Dev Dyn 2004;229:907–14.

Dixon J, Edwards SJ, Anderson I, Brass A, Scambler PJ, Dixon MJ. Identification of the complete coding sequence and genomic organization of the Treacher Collins syndrome gene. Genome Res 1997;7:223–34.

Dixon J, Jones NC, Sandell LL, Jayasinghe SM, Crane J, Rey JP, et al. Tcof1/Treacle is required for neural crest cell formation and proliferation deficiencies that cause craniofacial abnormalities. Proc Natl Acad Sci USA 2006;103:13403–8.

Donehower LA, Harvey M, Slagle BL, McArthur MJ, Montgomery Jr CA, Butel JS, et al. Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. Nature 1992;356:215–21.

Emes RD, Ponting CP. A new sequence motif linking lissencephaly, Treacher Collins and oral–facial–digital type 1 syndromes, microtubule dynamics and cell migration. Hum Mol Genet 2001;10:2813–20.

Franceschetti A, Klein D. The mandibulofacial dysostosis; a new hereditary syndrome. Acta Ophthalmol 1949;27:143–224.

Gerlitz G, Darhin E, Giorgio G, Franco B, Reiner O. Novel functional features of the Lis-H domain: role in protein dimerization, half-life and cellular localization. Cell Cycle 2005;4:1632–40.

Gorlin RJ, Cohen MM, Levin LS. Syndromes of the head and neck. Oxford, UK: Oxford University Press; 1990.

Hayano T, Yanagida M, Yamauchi Y, Shinkawa T, Isobe T, Takahashi N. Proteomic analysis of human Nop56p-associated pre-ribosomal ribonucleoprotein complexes. Possible link between Nop56p and the nucleolar protein treacle responsible for Treacher Collins syndrome. J Biol Chem 2003;278:34309–19.

Isaac C, Marsh KL, Paznekas WA, Dixon J, Dixon MJ, Jabs EW, et al. Characterization of the nucleolar gene product, treacle, in Treacher Collins syndrome. Mol Biol Cell 2000;11:3061–71.

Jones NC, Lynn ML, Gaudenz K, Sakai D, Aoto K, Rey JP, et al. Prevention of the neurocristopathy Treacher Collins syndrome through inhibition of p53 function. Nat Med 2008;14:125–33.

Levine AJ. p53, the cellular gatekeeper for growth and division. Cell 1997;88:323–31.

Marsh KL, Dixon J, Dixon MJ. Mutations in the Treacher Collins syndrome gene lead to mislocalization of the nucleolar protein treacle. Hum Mol Genet 1998;7:1795–800.

Noden D. The role of the neural crest in patterning of avian cranial skeletal, connective, and muscle tissues. Dev Biol 1983;96:144–65.

Phelps PD, Poswillo D, Lloyd GA. The ear deformities in mandibulofacial dysostosis (Treacher Collins syndrome). Clin Otolaryngol 1981;6:15–28.

Poswillo D. The aetiology and pathogenesis of craniofacial deformity. Development 1988;103:207–12.
Rubbi CP, Milner J. Disruption of the nucleolus mediates stabilization of p53 in response to DNA damage and other stresses. EMBO J 2003;22:6068–77.
Splendore A, Jabs EW, Felix TM, Passos-Bueno MR. Parental origin of mutations in sporadic cases of Treacher Collins syndrome. Eur J Hum Genet 2003;11:718–22.
Sulik K, Cook C, Webster W. Teratogens and craniofacial malformations: relationships to cell death. Development 1988;103:213–32.
Sulik KK, Johnston MC, Smiley SJ, Speight HS, Jarvis BE. Mandibulofacial dysostosis (Treacher Collins syndrome): a new proposal for its pathogenesis. Am J Med Genet 1987;27:359–72.
Treacher Collins E. Case with symmetrical congenital notches in the outer part of each lower lid and defective development of the malar bones. Trans Opthalmol Soc UK 1900;20:90.
Treacher Collins Syndrome Collaborative Group. Positional cloning of a gene involved in the pathogenesis of Treacher Collins syndrome. Nat Genet 1996;12:130–6.
Valdez BC, Henning D, So RB, Dixon J, Dixon MJ. The Treacher Collins syndrome (TCOF1) gene product is involved in ribosomal DNA gene transcription by interacting with upstream binding factor. Proc Natl Acad Sci USA 2004;101:10709–14.
Wiley MJ, Cauwenbergs P, Taylor IM. Effects of retinoic acid on the development of the facial skeleton in hamsters: early changes involving cranial neural crest cells. Acta Anat (Basel) 1983;116:180–92.
Wise CA, Chiang LC, Paznekas WA, Sharma M, Musy MM, Ashley JA, et al. TCOF1 gene encodes a putative nucleolar phosphoprotein that exhibits mutations in Treacher Collins syndrome throughout its coding region. Proc Natl Acad Sci USA 1997;94:3110–5.