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SYMPOSIUM: TROPHOBLAST DEVELOPMENT
REVIEW

The role of transcription factor Tcfap2c/TFAP2C in trophectoderm development

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Dr Schorle is head of the Department of Developmental Pathology at the Institute of Pathology, Bonn University Medical School, Germany. He completed his PhD in Würzburg, Germany working on establishment and analysis of interleukin-2-deficient mice. He received his postdoctoral training at the Whitehead Institute of Biomedical Research/MIT with Prof Dr R Jaenisch. While establishing Tcfap2c-deficient mice, he became interested in trophoblast biology and placenta development. The current members of his group, Peter Kuckenberg and Caroline Kubaczka, are involved in projects regarding early lineage decision and programming/reprogramming of the trophoblast.

Abstract In recent years, knowledge regarding the genetic and epigenetic programmes governing specification, maintenance and differentiation of the extraembryonic lineage has advanced substantially. Establishment and analysis of mice deficient in genes implicated in trophoblast lineage and the option to generate and manipulate murine stem cell lines from the inner cell mass and the trophectoderm *in vitro* represent major advances. The activating enhancer binding protein 2 (AP2) family of transcription factors is expressed during mammalian development and in certain malignant diseases. This article summarizes the data regarding expression and function of murine *Tcfap2* and human *TFAP2* in extraembryonic development and differentiation. It also presents a model integrating *Tcfap2c* into the framework of trophoblast development and highlights the requirement of *Tcfap2c* to maintain trophoblast stem cells. With regard to human trophoblast cell-lineage restriction, the role of *TFAP2C* in lineage specification and maintenance is speculated upon. Furthermore, an overview of target genes of AP2 in mouse and human affecting placenta development and function is provided and the evidence suggesting that defects in regulating *TFAP2* members might contribute to placental defects is discussed.

KEYWORDS: early lineage specification, *Tcfap2a*, *Tcfap2c*, Transcription factor AP2, trophectoderm, trophoblast stem cells

Introduction

Life begins with combination of oocyte and sperm resulting in the totipotent zygote. Through differentiation and proliferation, the zygote gives rise to all cell types of an entire organism (Hemberger et al., 2009; Kelly, 1977). With each additional division of the zygote, the developmental potential decreases leading to the first cell-lineage segregation at the blastocyst stage. The multipotent trophectoderm (TE) represents the extraembryonic trophoblast lineage that envelopes the pluripotent inner cell mass (ICM) (Rossant, 2007). Both lineages differentiate along their defined fate accompanied by the gradual loss of developmental potential. In the end, cells of the ICM give rise to

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all somatic tissues and the primitive endoderm, whereas the TE contributes to the extraembryonic tissue of the placenta. The specification and maintenance of ICM- and TE-committed cells, some of which harbour stem cell potential, is tightly controlled by extrinsic environmental signals and by the intrinsic transcriptional and epigenetic profiles of the respective cell.

The fact that stem cells of the mouse blastocyst can be derived and maintained *in vitro* opens a means to gather insights into the cellular mechanisms of early lineage specification, maintenance and differentiation. Pluripotent embryonic stem cells (ESC) derived from the ICM are able to self-renew and differentiate into all embryonic tissues, including the germline *in vivo* and *in vitro*. However, they are not able to give rise to trophoblast derivatives (Beddington and Robertson, 1989; Evans and Kaufman, 1981).

Likewise, trophoblast stem cells (TSC) can be derived starting from the polar TE of the blastocyst and from the extraembryonic ectoderm of the early post-implantation conceptus (up to embryonic day 6.5) (Himeno et al., 2008; Tanaka et al., 1998). TSC exclusively recapitulate the developmental potency of their parental origin and contribute to all trophoblast cell types of the conceptus. When injected into the blastocyst, TSC can repopulate all cell types of the trophoblast lineage but are excluded from the embryo proper of the resulting chimeras (Rossant, 2008; Tanaka et al., 1998). *In vitro*, TSC self-renew when grown in mouse embryonic fibroblast conditioned medium supplemented with fibroblast growth factor 4 (FGF4). When FGF4 is removed from the medium, TSC differentiate into all cell types of the mature placenta including syncytiotrophoblast cells of the labyrinth, spongiotrophoblast cell derivatives and trophoblast giant cell subtypes (Hemberger et al., 2004; Natale et al., 2009; Simmons et al., 2007; Tanaka et al., 1998). When injected under the skin of nude mice, TSC form transient aggregates, which erode host vessels resulting in blood-filled lacunas, demonstrating that TSC maintain their invasive properties *in vivo* (Kibschull and Winterhager, 2006; Kibschull et al., 2004). Thus, ESC and TSC lines represent a valuable tool for investigating all aspects and properties of the somatic and extraembryonic trophoblast cell lineage (Chen et al., 2010; Rossant, 2007, 2008; Rossant and Tam, 2009; Rugg-Gunn et al., 2010; Zernicka-Goetz et al., 2009).

The role of Tcfap2s in early TE specification, determination and maintenance

Genetic networks guiding early lineage restriction

In recent years, studies in mice have highlighted the key elements required for restriction of extraembryonic versus embryonic cell fate. Blastomeres of a mouse embryo remain morphologically indistinguishable up to the 8-cell stage (Cockburn and Rossant, 2010). During transition from the 8–16-cell embryo, symmetric and asymmetric cell divisions produce two populations of cells that differ both in their positions and properties. Apolar inside cells preferentially give rise to the ICM and polar outside cells are fated to become TE (reviewed in Cockburn and Rossant, 2010; Sasaki, 2010). It is most likely that both processes, cell polarity and cell position, affect each other to selectively regulate the Hippo pathway (Nishioka et al., 2008, 2009; Sasaki, 2010). In outer blastomeres, Hippo signalling leads to TEAD4-mediated transactivation of specific transcription factors of the trophectoderm and results in lineage segregation at the blastocyst stage (Nishioka et al., 2008, 2009; and reviewed in Chen et al., 2010; Cockburn and Rossant, 2010; Gasperowicz and Natale, 2011; Roberts and Fisher, 2011; Senner and Hemberger, 2010; Zernicka-Goetz et al., 2009).

The family of activating enhancer binding protein 2 (AP2) transcription factors is found throughout a variety of species (Eckert et al., 2005). In mouse and human, five different AP2 genes, named *Tcfap2a–e* or *TFAP2A–E*, respectively, are known. Upon dimerization they bind to GC-rich sequences and mediate transcriptional activation or repression (Eckert et al., 2005). In early mouse development, *Tcfap2a*, *Tcfap2b*, *Tcfap2c* and *Tcfap2e* transcripts can be detected in oocytes (Winger et al., 2006). During further developmental progression, *Tcfap2a* and *Tcfap2c* become restricted to the TE at blastocyst stage, while *Tcfap2b* and *Tcfap2e* are no longer detected (Auman et al., 2002; Kuckenberg et al., 2010; Winger et al., 2006). In self-renewing TSC, TCFAP2C and TCFAP2A proteins are highly expressed (Kuckenberg et al., 2010; Winger et al., 2006). During differentiation of TSC, expression of TCFAP2A and TCFAP2C is maintained and expression of TCFAP2B is induced (Kuckenberg et al., 2010). Of note, while Winger et al. (2006) detected a weak signal for *Tcfap2e* RNA, Kuckenberg et al. (2010) was not able to detect TCFAP2E protein in differentiating TSC cultures. This expression pattern suggests a function for *Tcfap2a* and *Tcfap2c* in the TE-lineage identity (i.e. specification, maintenance and/or differentiation). Establishment and analysis of murine knockout models demonstrated that mice deficient for either *Tcfap2a* or *Tcfap2b* develop a functional placenta but display deficiencies in the cranial neural crest (*Tcfap2a*) (Schorle et al., 1996; Zhang et al., 1996) or failure of kidney function (*Tcfap2b*) (Moser et al., 1997). This indicates that *Tcfap2a* and *Tcfap2b* are not essential for development of extraembryonic cells.

However, *Tcfap2c*-knockout mice embryos die at embryonic day 7.5. Lack of *Tcfap2c* is associated with a reduction in the proliferation of the TE compartment leading to fewer ectoplacental cone and trophoblast giant cells (Auman et al., 2002; Werling and Schorle, 2002). Of note, combined loss of both *Tcfap2a* and *Tcfap2c* led to an earlier lethality than the loss of *Tcfap2c* alone (Winger et al., 2006) suggesting that these two AP2 genes have redundant functions in TE. Unfortunately, morphological analyses of double deficient placentas were not performed. In addition TCFAP2C might form homo- or heterodimers (with TCFAP2A), which could modulate the binding of TCFAP2C to target gene promoters (Williams and Tjian, 1991).

These data suggest that the specification of TE occurs in the absence of *Tcfap2c* since the zygote proceeds to a functional blastocyst and implants into the uterus. This is in contrast to the phenotypes obtained by deleting *Tead4* and *Cdx2*, which are essential for TE specification. For example, embryos deficient in the Hippo pathway target gene *Tead4* were not capable of blastocyst cavitation (Nishioka et al., 2008; Wu et al., 2010; Yagi et al., 2007). Also, deletion of the *Tead4* target gene, the caudal related homeobox-factor *Cdx2*, resulted in inhibited implantation. Interestingly, the *Cdx2*-deficient embryos initiate TE specification since