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Intercellular bridges are essential for human parthenogenetic cell survival

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ABSTRACT

Parthenogenetic cells, obtained from *in vitro* activated mammalian oocytes, display multipolar spindles, chromosome malsegregation and a high incidence of aneuploidy, probably due to the lack of paternal contribution. Despite this, parthenogenetic cells do not show high rates of apoptosis and are able to proliferate in a way comparable to their biparental counterpart. We hypothesize that a series of adaptive mechanisms are present in parthenogenetic cells, allowing a continuous proliferation and ordinate cell differentiation both *in vitro* and *in vivo*. Here we identify the presence of intercellular bridges that contribute to the establishment of a wide communication network among human parthenogenetic cells, providing a mutual exchange of missing products. Silencing of two molecules essential for intercellular bridge formation and maintenance demonstrates the key function played by these cytoplasmic passageways that ensure normal cell functions and survival, alleviating the unbalance in cellular component composition.

1. Introduction

Parthenogenesis is a form of asexual reproduction in which an embryo develops without sperm contribution from an unfertilized egg. It naturally occurs in several invertebrate species, including nematodes, water fleas, aphids, bees, and in few vertebrates, such as fish, amphibians, and reptiles, but not in mammals. However mammalian mature oocytes can be activated *in vitro*, leading to the generation of parthenotes that will form blastocysts morphologically indistinguishable from those derived from fertilized eggs. Despite this similarity, many studies reported fundamental alterations, which are surprisingly compatible with the survival of cells and do not affect the formation of fetuses that can develop to different stages after activation, depending on the species (Hipp and Atala, 2004).

Indeed, the high degree of homozygosity, as well as the loss of imprinting, which are inevitable consequences of parthenogenesis, would be a possible cause of malignant transformation (Tuna et al., 2009) and cancer development (Jelinic and Shaw, 2007). Another aspect that has recently received particular attention is the high incidence of aneuploidy reported in different species. Extensive karyotype analyses of pre-implantation mammalian parthenotes showed that the

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majority display chromosomal abnormalities, including human (Combelles et al., 2011; Delhanty, 2005), sheep (Alexander et al., 2006), pig (Somfai et al., 2006) and bovine (Bhak et al., 2006; Van De Velde et al., 1999; Winger et al., 1997).

We recently demonstrated that these defects persist in cell lines derived from human and pig parthenogenetic blastocysts as well as in fibroblasts derived from sheep post-implantation embryos (Brevini et al., 2012) and are caused by the absence of the paternal centriole. In all these species, in fact, differently from the mouse, the centrosomes of the male and female gametes undergo a reciprocal reduction, so that, at the time of fertilization, the proximal centriole is carried into the ooplasm by the sperm, where it mixes with a stockpile of maternal centrosomal proteins and generates a functional zygotic centrosome (Manandhar et al., 2005; Schatten and Sun, 2009; Sun and Schatten, 2007). The lack of paternal centriole in the activated oocyte would lead to the perturbation of a regulatory mechanism driving centriole de novo assembly and the subsequent formation of multipolar spindles, resulting in chromosome malsegregation and aneuploid cell divisions (Acilan and Saunders, 2008; Delattre and Gonczy, 2004).

Surprisingly those alterations are not accompanied by a correspondingly high rate of apoptosis, but conversely, parthenogenetic cell lines are able to proliferate in a way comparable to their biparental counterpart (Brevini et al., 2012).

We hypothesize that a series of adaptive machineries are present in parthenogenetic cells, allowing the continuous proliferation and ordinate differentiation described both *in vitro* (Isaev et al., 2012; Turovets et al., 2011) and *in vivo* (Kure-bayashi et al., 2000; Loi et al., 1998). One of the possible mechanisms may be represented by the formation of stable intercellular bridges. These structures are formed during cell division in certain tissues and/or during specific developmental stages, where the cytokinesis process is incomplete and are manifested by arrest of cleavage furrows, giving rise to cells interconnected in syncytia (Burgos and Fawcett, 1955; Greenbaum et al., 2011; Robinson and Cooley, 1996, 1997; Vidulescu et al., 2004). This evolutionarily conserved mechanism has been extensively described both in the female and male germline, in species ranging from Drosophila to humans (Fawcett et al., 1959). At the moment, the best characterized gene involved in intercellular bridge formation and stabilization is *Testis Expressed Gene 14* (*TEX14*). Its synthetized protein has been localized in germ cell intercellular bridges (Greenbaum et al., 2006) and shown to act by blocking cell abscission and leading to transformation of midbodies into intercellular bridges (Greenbaum et al., 2007; Iwamori et al., 2010).

It has been demonstrated that the bridges display a diameter range of ~0.2–10 μm (Haglund et al., 2011) and join the cytoplasm of neighboring cells, allowing the passage of mRNAs, proteins, mitochondria and ribosomes (Greenbaum et al., 2011; Hermo et al., 2010). These mutual exchanges between cells are made possible thanks to the expression of *Translin* (*TSN*) a gene known to modulate mRNA transport and translation in male germ cells as well as in neurons (Stein et al., 2006; Yang et al., 2003).

Several roles for mammalian intercellular bridges have been proposed. The most supported one suggests they may be necessary for haploid cells, such as germ cells, to remain "phenotypically diploid" after meiosis (Braun et al., 1989; Erickson, 1973; Fawcett et al., 1959).

Based on these observations we investigated the presence of structures that may provide a mutual exchange of missing products in parthenogenetic cells, alleviating the unbalance in cellular component composition that would hamper normal cell functions. In this manuscript we demonstrate the presence of intercellular bridges that establish a wide communication network among human parthenogenetic (HP) cells and allow intercellular trafficking. We use microinjection of a gap junction-nonpermeant tracking molecule to show that these bridges display functional activity, allowing large molecules to be exchanged and shared among cells. We transfect specific silencing RNAs, targeted against molecules that play a role in intercellular bridge formation and maintenance, to investigate whether these structures play an essential role in ensuring cell functional integrity.

2. Materials and methods

All chemicals were purchased from Sigma (Italy) unless otherwise indicated.

2.1. *Human parthenogenetic cell line culture*

HP cell lines used in these experiments were derived from parthenogenetic blastocysts (Paffoni et al., 2007) and cultured in DMEM (Invitrogen) supplemented with 10% Knockout serum replacer (Invitrogen), 5% fetal bovine serum (Invitrogen), 1 mM glutamine, 0.1 mM β-mercaptoethanol, 5 ng/ml human recombinant basic Fibroblast Growth Factor (R&D System) and 1% nonessential amino acid stock (Invitrogen) (Brevini et al., 2009, 2012). Experiments were performed on cells at a passage comprised between 5th and 20th.

2.2. *Ultrastructural and optical analysis*

HP cells were collected for light microscopy, transmission electron microscopy (TEM) and scanning electron microscopy (SEM) at different time points: before siRNA treatment (T0) and at 12 and 24 hours siRNA post-transfection. For TEM analysis, samples were fixed for 1 hour in 2% glutaraldehyde in 0.1 M Na-cacodylate buffer (pH 7.2). Specimens were washed in 0.1 M Na-cacodylate buffer (pH 7.2) and were post-fixed at 4 °C for 1 hour with 1% osmic acid in cacodylate buffer (pH 7.2). After standard dehydration in ethanol series, samples were embedded in an Epon-Araldite 812 mixture and sectioned with a Reichert Ultracut S ultratome (Leica, Nussloch). Semithin sections were stained by conventional methods and were observed with a light microscope (Olympus). Thin sections were stained by uranyl acetate and lead citrate and observed with a JEOL JEM-1010 transmission electron microscope (JEOL Ltd.). For SEM, cells were fixed and dehydrated as described above, then treated with hexamethildisilazane and mounted on polylysinated slides, air dried and subsequently covered with a 9 nm gold film by flash evaporation of carbon in an Emitech K 250 sputter coater (Emitech). Specimens were examined with a SEM-FEG Philips XL-30 microscope (Philips).