Abstract

Female germ cells (also known as oocytes) from mammalian species are found in the ovaries in a state of meiotic arrest at prophase I. It is not until puberty that oocytes start to be selected to grow, overcome their meiotic arrest and ovulate, so they become capable of being fertilized and give rise to new individuals. Half of the genetic information from these new individuals comes directly from the oocyte itself, therefore, oocyte and meiosis quality are of great importance for the reproduction.

One of the factors, which can drastically reduce oocyte quality in several mammalian species is the advanced age of females. In both mice and humans age-related poor oocyte quality is reflected by a large increase of chromosomal aneuploidy rates. Having an incorrect number of chromosomes reduces embryo viability and may cause severe clinical outcomes. The work of this thesis was primarily directed towards a better understanding of the causes behind age-related aneuploidy in mice oocytes.

One of the most characteristic features of oocytes is the fact that they become transcriptionally silent after meiotic resumption, relying heavily on translational control for protein expression. Here we show that after nuclear envelope break down (NEBD), one of the main protein kinases regulating translational initiation, mTOR becomes highly active and as a response 4E-BP1, one of its substrates is inactivated. This documents the tight connection between NEBD and timely initiation of translation.

Our results show that oocytes from aged females (AF, 12 months old) have faster meiotic progression than oocytes from young females (YF, 2 months old). AF oocytes underwent NEBD and polar body (PB) extrusion 30 minutes earlier than YF oocytes, accompanied by a faster phosphorylation of the nuclear lamina component LAMIN A/C. Accordingly, the results of our further experiments revealed an increased activity of maturation promoting factor (MPF), the main regulator of meiotic maturation, in AF oocytes.

Furthermore, we adapted a polysomal profiling method, which allowed us to extract RNAs bound to polysomes (therefore more prone to be translated) from post-NEBD oocytes of both maternal age groups followed by next generation sequencing. Utilizing this technique we were able to reveal a considerable amount of transcripts, which were differentially translated between YF and AF. We validated these differences on protein level at the post-NEBD stage of choosing four most interesting proteins in both age groups: SGK1, CASTOR1, AIRE and EG5. All these four proteins were localized at the spindle region. Moreover, when the levels/activity of SGK1 and CASTOR1 were altered in YF oocytes, we detected significant defects in chromosome alignment and cytokinesis, which supports a possible role of these proteins in age-related aneuploidy.

Altogether, our results show that the post-NEBD stage is the fundamental starting point, in which oocytes from YF and AF start to manifest their differences basically through differential translation of specific RNAs. These differences in the translation of these specific RNAs could be then involved in the higher aneuploidy rates seen in AF oocytes, since our results show that the alteration of their expression or activity can lead to cytokinetic abnormalities.