ABSTRACT

PUM proteins are post-transcriptional gene expression regulators involved in morphogenesis and gametogenic cell development of various organisms. Their RNA-binding domain called PUF recognizes the UGUANAUA motif named PBE (Pumilio Binding Element), located mostly in the 3'UTR of mRNAs. Upon binding, PUM recruits additional proteins, such as NANOS that play the role of co-factors in that process. Composition of the resulting ribonucleoprotein (RNP) complex built on target mRNA determines whether it undergoes translational activation, repression leading to storage or degradation. That type of posttranscriptional regulation ensures an appropriate level of individual proteins in the cell and contributes to homeostasis. The general objective of this dissertation was to reveal significance of PUM1 and PUM2 paralogue in that process in the context of human reproduction, based on TCam-2 cell line originating from seminoma, representing male germ cells at an early stage of prenatal human development. To do so three specific issues were addressed. 1/ What is the influence of PUM1 and PUM2 on processes crucial for germ cell development, such as: apoptosis, cell division and cell cycle, by modulating the level of mRNA targets. These processes are of a high importance, since their malfunction may cause infertility, as well as the germ cell tumours. 2/ The mechanism of repression by PUM proteins in context of particular protein cofactors, as well as, 3/ functional differences between PUM1 and PUM2.

By applying RNA immunoprecipitation, RT-qPCR and reporter luciferase tests, a novel mRNA target encoding kinesin KIF18A which is repressed by PUM1 and PUM2 was identified. Moreover, that repression was mediated by 3'UTR containing PBE motifs. Furthermore, by using MTS test and flow cytometry it was shown that PUM1 and PUM2 by repressing mRNA KIF18A, modulated proliferation, apoptosis and the TCam-2 cell cycle. Importantly, KIF18A induced effects opposite to PUM1 and PUM2, as its siRNA silencing caused a decrease of TCam-2 cells proliferation, increase of apoptosis and an arrest in G2/M phase of the cell cycle. Studies on two other novel PUM targets encoding SPIN1 and SPIN3 demonstrated that SPIN1 plays a role of a proto-oncogene, while SPIN3 of a tumour suppressor. Namely, overexpression of SPIN1 caused a decrease of apoptotic cells, while SPIN3 overexpression induced an opposite effect. As it was shown by luciferase test, PUM1 and PUM2 overexpression caused repression of SPIN1 and SPIN3 mRNAs, mediated by their 3'UTR. Also, PUM1 alone, unlike PUM2, strongly stimulated apoptosis and slightly downregulated the cell cycle. That may indicate that PUM1 acts as a tumour suppressor, similarly to SPIN3. Therefore, PUM1 may play a role of tumour suppressor by the regulation of apoptosis and the cell cycle. Thus, repression of