

6.1 The human ODZ4 Full-length Transcripts

Many ODZ4 transcripts that lie within the ODZ4 locus have been submitted to genomes database recently. However, currently there are no evidences supporting that the ODZ4 predicted full-length transcript exists in humans. For example, the UCSC Genome Browser [105] includes several transcripts but none span the overall length of the predicted ODZ4 mRNA. A large part of these described transcripts are concentrated on the 3' or the 5'-end, and most of them, have been found as EST sequences during transcript screening performed by different consortia working on generating human full-length cDNAs [115-119]. The other ODZ4 sequences present on the UCSC Genome database derive from direct submissions. A current representation of ODZ4 transcripts in the UCSC Genome Browser is depicted in Figure A1.

Here, we demonstrated for the first time that human ODZ4 transcript can be continuously expressed from base position number 345 to base position number 9961, encompassing all the protein-coding exons in SKOV3 and MCF7 human cancer-derived cell lines. Furthermore, we found that in these cell types, an insert splice variant of the partial full-length ODZ4 transcript, identified during this work, also expresses all protein-coding exons and it can be detected under particular conditions of RT-PCR amplification. Additionally, our findings suggest that others ODZ4 transcripts may be present and could be generated from a complex mode of splicing processes.

As previously mentioned, there is no pre-existing data concerning the expression of exon 4 in humans, except a report from Wakamatsu (2007) where the expression of exon four was found as EST sequence at cDNA library of subthalamic nucleus tissue (GenBank Accession n. AK309124) [121]. The region comprised from exon 2 to exon 4 in AK309124 shares 100% of

sequence identity respect to the ODZ4 RefSeq transcript. However, no additional information regarding 5'-end rather than these was found regarding the human ODZ4 transcript.

Wang et al. (1999) evidenced that the gamma-heregulin (Y-HRG), a chimeric protein generated by a fusing translocation of the heregulin and the ODZ4 genes is found in MDA-MB-175 breast carcinoma cell lines. Since the Y-HRG transcript region enclosed by the exon 3 to 10 is identical to the ODZ4 transcript region encompassed by the exon 3 and 12, we performed a screening for Y-HRG transcript in MCF7 and SKOV3 cell lines. Accordingly to the Wang report, no RT-PCR product was found in these cell lines [108] (data not shown). Since interference with Y-HRG transcript was not detected, our findings are referred only to the ODZ4 transcripts.

6.2 The ODZ4 Splice Variants

Accordingly to our findings (Figure 6.1), different ODZ4 splice variants generated by the “canonical” promoter have been detected during mice embryo development. These spliced variants shown a diverse exon expression pattern depending on development stage and type of tissue. Additionally, it was also evidenced that an alternative promoter may generate distinct alternative transcript forms that were differentially expressed in some specific tissues. For example, the murine exon 2 generates 3'-truncated variants that can be detected by RT-PCR only in the brain or only in the ovary whereas others exon-2 derived transcripts can be detected in almost all adult tissues [47].

Moreover, within the insert region enclosed by exon 4 and 8 primer pairs, some particularities were noted: three clones showed three different types of insert sequences ranged between 210, 211 and 212 bp generating a PCR-product of the 1177, 1178 and 1179bp in

length, respectively (Figure 6.2). However, only the insert of 212bp might be an *in-frame* ODZ4-spliced variant.

As result, we found no clear data regarding how many transcripts are expressed in these human cell lines, which of these messenger will be translated or which could be the transcripts that may be associated to ovarian cancer manifestation. Nevertheless, these issues will be addressed in future outcomes during the project.

6.3 Human ODZ4 Transcripts In Ovarian and Breast Cancer-derived Cell Lines

Based on the experimental results, we propose that the predicted structure for the ODZ4 full-length is presents within SKOV3 and MCF7 cell lines, however it would be a minor form expressed by these cell lines. Since as observed in sections 5.5 and 5.6.2, were obtained respectively, a higher PCR-product yields and a stronger hybridization-signals that concerning principally the insert variant forms. These latter differ from the “canonical” transcript form by their major retardation on gel electrophoresis. An overview of our experimental findings is given in Figure 6.1.

It would be useful to carry out *In-situ hybridization* experiments to visualize which of these many transcripts posses a cytoplasmatic localization in these cell lines. The *in vitro* obtained expression pattern may reflect those transcripts that are been actively processed for the translation. Eventually, these observations can be translated to an *in vivo* context in the frozen-tumor biopsies in order to investigate if some differences in the ODZ4 transcript expression among the ovarian tumor cells exist.

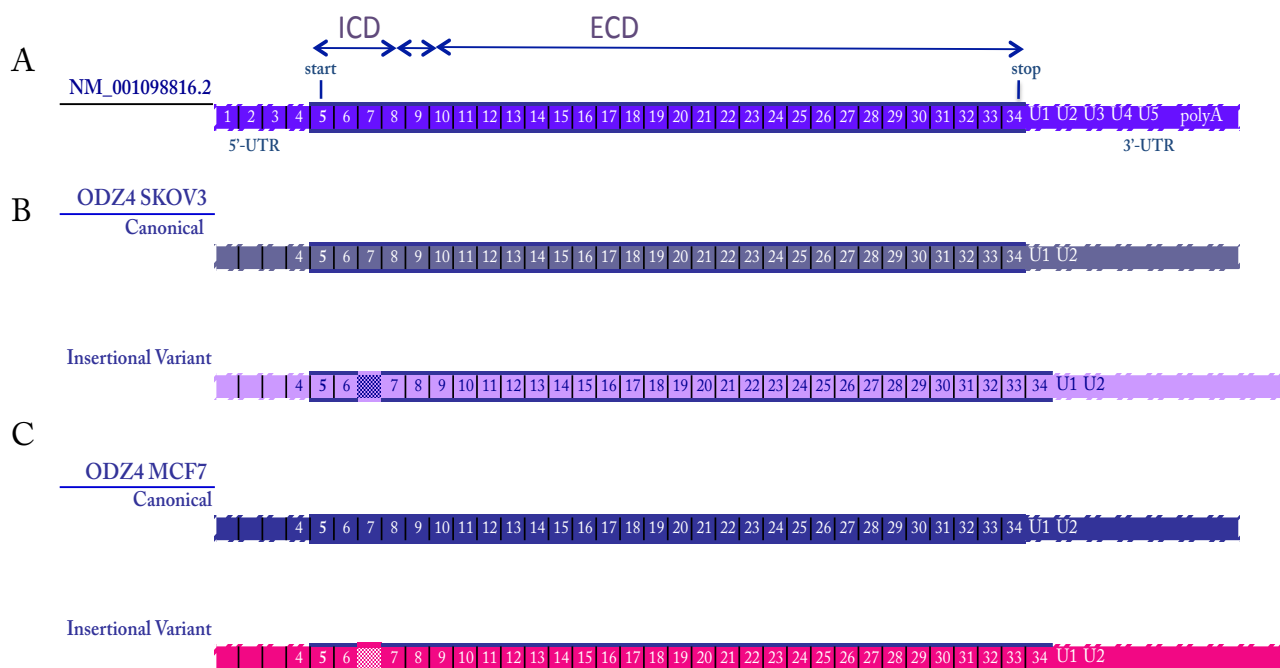


Figure 6.1. ODZ4 partial-full length transcript in human ovarian and breast cancer-derived cell lines. The figure represents the structural conformations of the ODZ4 messengers derived from ovarian (SKOV3) and breast (MCF7) cancer-derived cell lines characterized by RT-PCR experiments. The exon-expression pattern was examined gradually until the 3'-end of predicted ODZ4 sequences was reached. An insertional splice variant was identified in both cell lines.. A) The predicted structure of ODZ4 mRNA in the RefSeq GenBank. B) Characterization of the ODZ4 transcripts found in SKOV3 cell line. C) Characterization of the ODZ4 transcripts found in MCF7 cell line. ICD: Intracellular domain, ECD: extracellular domain. Fill boxes indicate the insert fragment in splice ODZ4 variant. Numbers within coloured boxes indicate a single exon. The lack of numbers in the UTR regions implies that these portions await further investigations.

We intend to achieve functional studies for teneurin-4. To this end, the partial-ODZ4 full length (e.i the transcript region enclosed by exon 4 to U1 region) will be cloned and subsequently transfected to evaluate its expression effect on the cisplatin-based drug resistance assay, as well as during angiogenesis and apoptosis responses in ovarian and breast human cancer cell lines that do not express the ODZ4 transcript. HeLA and MDA-MB237 tumor cell lines may be good candidates for this approach.

6.4 Final Conclusion

Our interests were to shed light into the structural organization of the protein coding and non-coding regions of the ODZ4 transcript in human cancer-derived cell lines as initial approach towards the elucidation of its role *in-situ*. Thus, in this study, which was aimed at deciphering the structural conformation of the ODZ4 transcript, we intended to generate new molecular knowledge that could be relevant to the study of functional role of ODZ4 in human cancers.

(N.A) During the manuscript writing new evidence regarding human ODZ4 was published but it was not included here [122].



Figure 6.2. Genomic insert-sequences found in ODZ4 mRNA derived from ovarian cancer cell line (SKOV3). Sequencing of the cDNA region encompassed by exon 4 and 8 primer pairs showed three different types of genomic-insert sequences that lie between exons 6 and 7. Arrows indicates the relative position of primers annealing. A) 212 insert nucleotides generate a PCR-product of 1179 bp in length. Acc.n. HE601756. B) 211 insert nucleotides generate a PCR-product of 1178 bp in length. Acc.n. HE601755. C) 210 insert nucleotides generate a PCR-product of 1177 bp in length. Acc.n HE601754.