To prevent carcinoma caused by RCC1-mutation through drugs via RNA assay

by Marie Köhler at 15.02.2021

Introduction

A human body has grown over time due to cell division. The cell cycle allows to grow and to renew cells, but it can also lead into diseases once the cell cycle was not completed properly.

The cell cycle is separated into 5 sections – (G_0 -,) G_1 (gap) -, S (synthesis)-, G_2- and M (mitose)-phase. Cells are not always in cell division. Are the cells dormant, they are in phase G_0. The actual replication of the chromosomes happens from G_1- to G_2-Phase (called interphase). The mitose-phase (M-phase) is again divided into 4 sections – prophase, metaphase, anaphase, and telophase, where the chromosomes get divided into two cells [Vermeulen et. al., 2003].

The cell cycle has three major control points (in G_1-, G_2- and M-phase) to ensure the correct DNA-replication. The cell cycle and the control points are dependent from different cyclins [Barnum et. al., 2014].

The cell cycle is also dependent of a Regulator of Chromosome Condensation 1 (RCC1), which is a critical cell cycle regulator. A lot of proteins contain one or more RCC1-like domains (RLD's) which can be divided into five subgroups dependent on their biochemical structure [Hadjebi et. al., 2008].

From former studies, RCC1 has multiple functions as a guanine nucleotide exchange factor on small GTP-binding proteins [Klebe et. al., 1995], enzyme inhibition and interactions with proteins and lipids [Hadjebi et. al., 2008]. Therefore, RCC1 can bind on histones H2A/H2B, the nucleosome and less likely the DNA itself. Once RCC1 has bound on the histone-complex, it is able to phosphorylate RanGDP to RanGTP, which is necessary for further biochemical pathways needed for cell division [Zierhut et. al., 2015].

Is RCC1 not available due to mutations, DNA will be transferred wrongly into mitosis. Also, it will cause a spindle positioning defect [Carazo-Salas et. al., 1999] and a chromosome segregation [Li et. al., 2004]. This is going to cause cancer [Moore et. al., 2002] like e.g. breast cancer [Riahi et. al., 2018] or neuroplastoma (RRID:CVCL V008).

Since there are some questions about where mutations in the RCC1-gen occur, we will do further studies to find out whether there is a way to prevent patient to get diseases like cancer.

Results

All Sequences to RCC1 are found in Ensembl under the Accession number ENSG00000180198. RCC1 has some isoforms which are available there, too. The counts of base-pairs (bp) are from 481 bp to 2844 bp.

In this paper the results of the largest RCC1-form (ENST00000373833.10) is shown. It has a length of 2844 bp, has a translation length of 421 residues and has each 13 Introns and Exons while 9 of 13 Exons are coding.

In figure 1 the gene model is introduced:

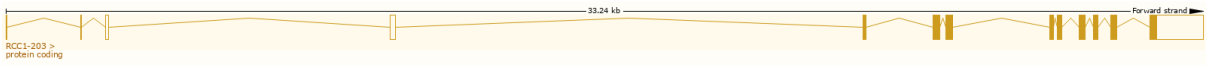

Figure 1 Gene model of RCC1's exons and introns. The exons are block-shaped, while the coding exons-blocks are colored in. The introns are small lines between the exons. 5'UTR is at the left site of the figure, 3'UTR at the right site. Figure found at Ensembl

From the programming tool "R" it can be figured out, that the 3'UTR has a total of 1294 amino acids (aa), while the 5'UTR has 285 aa.

To find the open reading frames (ORF) of the protein, NCBI ORFfinder is used. Different examples of ORF's of the complete genome are shown in this table:

Table 1 ORF of RCC1 (complete genome sequence). Different start codons in comparison with different minimal ORF lengths were used. Found by NCBI ORFfinder

ORF start codon	Minimal ORF length [nt]	ORF found
'ATG' only	75	224
'ATG' only	150	83
'ATG' + alternative initiated codons	75	541
'ATG' + alternative initiated codons	150	212

Since the function of RCC1 is to control the transition into the mitosis, RCC1 is expressed in all cells. Thereby the experiments (ENSG00000180198) figured out, that RCC1 is highly expressed in embryos, neonates and infants. The expression of RCC1 in cells reduces in older age.

Due to the experiment "32 Uhlens Lab", there is a high expression in organs like lymph node (54 TPM (Transcripts Per Kilobase Million)), testis (59 TPM) and tonsil (51 TPM). The expression is reduced e.g. in the bone marrow (34 TPM), colon (28 TPM) or spleen (24 TPM). The lowest expression was found in the heart and the skeleton muscle tissue with each 2 TPM.

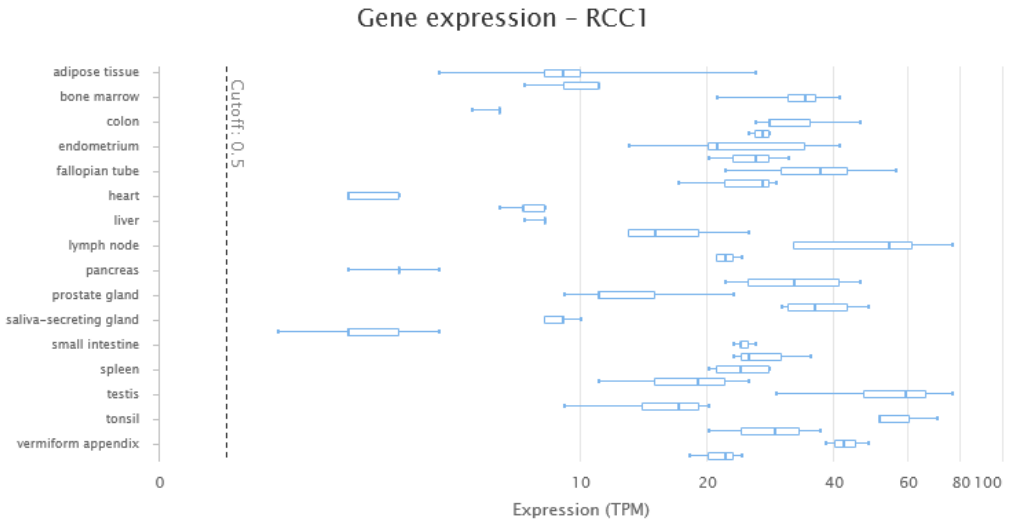

Figure 2 Expression of RCC1 in different organs in TPM. (Expression Atlas, 32 Uhlens Lab)

Next to the expression of RCC1 itself, it has been experimentally shown that's there are at least 50 similar expressed genes. Those genes are located on different Chromosomes, but they are also expressed in all tissue and take place in regulating the cell development.

When comparing 21 genes out of 50 similar expressed genes (e.g. ALS2, HERC, RCCD1, RCC2, RCCL1, AREL1, RCBTB), the program DAVID shows a.o., that there is a 61,9 % match in biological process as the function in macromolecule modification, followed by protein modification- and cellular protein metabolic process (57,1 %), regulation of GTPase activity (38,1 %) to chromosome segregation and mitotic nuclear division (14,3 %).

It also shows, that there are similarities of cellular component e.g. in the intracellular part (95,2 %), cytoplasm (85,7 %), nucleus (57,1 %), cytosol (38,1 %), cytoskeleton (28,6 %) and centrosome (19,0 %).

In comparison to the molecular function, DAVID shows e.g. a 42,9 % similarity for transferase activity, followed by the molecular function regulator (38,1 %), enzyme binding (23,8 %) to protein kinase binding (14,3 %).

When comparing the human RCC1 to RCC1 from the Abingdon island giant tortoise (ENSCABT00000014342.1), Bolivian squirrel monkey (ENSSBOG00000024509.1) and Rabbit (ENSOCUT00000001170.4) by Needleman-Wunsch- and Smith-Waterman-Algorithm, it is shown that the Abingdon island giant tortoise has the highest identity and similarity (~ 78,0 % for transcript and CDS; up to 93 % for protein) to the human RCC1-gen. It is followed by the Rabbit (~ 40 % for transcript and CDS; up to 40 % for protein) and the Bolivian squirrel monkey (~ 40 % for transcript and CDS; up to 39 % for protein).

Besides, all genes have a difference length of amino acids. The Abingdon island giant tortoise has a length of 388 aa, the Bolivian squirrel monkey 278 aa and the Rabbit 495 aa. The human RCC1 has a total of 421 aa.

To compare the species itself, it was created a phylogenetic tree via "phyloT". It is shown that, in comparison to the whole genome, the Homo sapiens and the Bolivian squirrel monkey are the most similar.

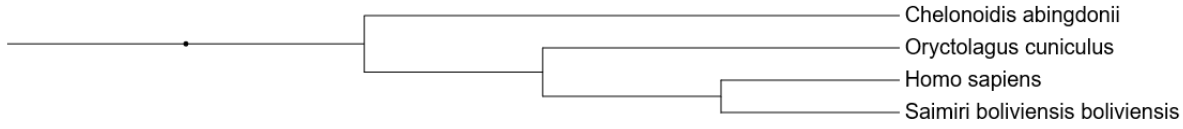

Figure 3 phylogenetic tree via phyloT. It is shown, that the Homo sapiens and the Saimiri boliviensis boliviensis (Bolivian squirrel monkey) are the most similar on a genetic base

Discussion

It were done four experiments, where three of them were associated with cancer (cell lung carcinoma, renal cell carcinoma (RRID:CVCL_WI01)). The experiments were done by micro assays, one as a DNA-assay (ChIP-seq) and two as an RNA-assay (transcription profiling by array). There are no homologues of RCC1 in other species.

Since we know, that a mutation in the RCC1-gen leads into cancer, we need to prove why some cancers are more likely than others (e.g. renal cell carcinoma). As the experiments shown, the

expression levels of RCC1 in organs are different from each other. It could explain the increased chance of cancer for one specific organ. Due to the experiments of “32 Uhlens lab” kidneys have a reduced expression of RCC1 than others, but other experiments found out that renal cell carcinoma is quite common.

Though it seems like, the expression of RCC1 is increased in organs that are needed for the immune system. So, it does make sense, due to increased expression, that it is more likely to get cancer in those organs.

To come to further results, it would be needed to study where the mutation(s) is located and if there will be drugs who could prevent mutation. One way could be, to increase the mutation rate in the Abingdon island giant tortoise. Though we are genetically more familiar with the Bolivian squirrel monkey, we have huge differences in the length of the RCC1-gen. This probably causes into wrong results. Apart from an identity of 93% in the protein structure, the amount of the amino acid sequence in the Abingdon island giant tortoise is quite similar as well.

In case of not doing animal experiments, a cell culture with human cells can be used. Mutation can be inserted randomly through e.g. SMS. Another way is to let cells mutated through chemicals. There will be a chance to study first how cells are reacting by random mutation. It will show whether those cells can fix the mutations, do not really mind those mutation or lead into cancerous cells.

Those sequences can be aligned through BLAST or UniProt Align. A way to show the exact location of the mutation(s), ApE can be used.

Once mutation have been studied and compared, it will be necessary to gain statistical information. This can be investigated through RStudio (e.g. mutSignatures). To come to an end, a drug can be created to stop proliferating those mutated cells, combined with an antibody treatment for destroying the mutated cells.

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* Name invented for this purpose