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a methodological journal

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Journal of Integrated OMICS

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SPECIAL ISSUE

SELECTED ABSTRACTS OF THE 2ND INTERNATIONAL CAPARICA CONFERENCE IN SPLICING (SPLICING 2018)

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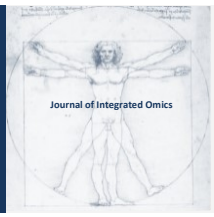

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE 2ND INTERNATIONAL CAPARICA CONFERENCE IN SPLICING (SPLICING 2018)

Splice variants of matricellular proteins in cancer

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Available Online: 30 August 2018

ABSTRACT

SPOCK (SPARC/Osteonectin, cwcv and kazal like domains proteoglycan) proteins are matricellular proteins involved in matrix remodelling through the regulation of metalloproteinases [1]. SPOCK1 and SPOCK3 have been reported to play a role in diverse cancer types. Upregulation of SPOCK1 in pancreatic cancer is associated with poor prognosis [2]. In contrast, SPOCK3 is expressed in normal brain tissues and is downregulated in glioma [3]. Alternative splicing is an important source of protein diversity, and changes in alternative splicing events are associated with a range of different diseases, including cancer [4]. In this study, we combined reverse transcriptase-polymerase chain reaction (RT-PCR) and western blot assays to identify multiple isoforms of SPOCK1 and SPOCK3 matricellular proteins with cell-type specific expression. At the mRNA level, SPOCK1 splice variants were found to be expressed in stromal cells and absent in pancreatic cancer cells. Multiple SPOCK3 splice variants were also expressed in stromal cells. It will be important to determine the role of these novel SPOCK isoforms in the regulation of matrix remodelling in pancreatic cancer.

Acknowledgments: We would like to thank colleagues who provided cell lines used in this study, and members of the laboratory for useful discussions.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE 2ND INTERNATIONAL CAPARICA CONFERENCE IN SPLICING (SPLICING 2018)

Modulation of mRNA alternative splicing of telomerase catalytic subunit hTERT by endonuclease EndoG in human colorectal cancer cells

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ABSTRACT

Telomerase activity is known to be regulated by alternative splicing (AS) of its catalytic subunit hTERT (human Telomerase Reverse Transcriptase) mRNA. Only full-length α+β+ hTERT is catalytically active. Induction of α+β– non-active spliced hTERT leads to inhibition of telomerase activity [1]. Expression of hTERT splice-variants strongly correlates with the expression on apoptotic endonuclease EndoG in human colorectal cancer cell lines [2]. The aim of the study was to determine the role of EndoG in the regulation of hTERT AS. Increased expression of α+β– splice variant, reduced expression of α+β+ full-length hTERT and inhibited telomerase activity was found in CaCo-2 cells after EndoG over-expression induced by pEndoG-GFP transfection. Treatment of CaCo-2 cell cytoplasm or naked nuclei with recombinant EndoG led to induction of α+β– hTERT mRNA. In order to investigate the origin of EndoG-produced splice-switching agent we digested total RNA from cell nuclei with recEndoG followed by nuclei incubation with digested RNA. RecEndoG-digested RNA could induce AS of hTERT that denotes EndoG-produced RNA oligonucleotide can induce AS in naked nuclei. Active 47-mer RNA oligonucleotide and its sequence was determined by hybridization with DNA oligonucleotides. Transfection of CaCo-2 cells with artificial 47-mer RNA oligonucleotide could induce AS. Using Next Generation Sequencing of total RNA, we identified long non-coding RNA, that is the precursor of 47-mer RNA oligonucleotide. Its size is 1754 nucleotides. The expression of non-coding RNA in cells was not depending from EndoG. Most likely, quantity of 47-mer RNA oligonucleotide is being regulated by EndoG activity, but not by the levels of non-coding RNA expression. In our work, we for the first time demonstrated that EndoG can regulate AS of hTERT and telomerase activity by producing small splice-switching RNA oligonucleotide.

Acknowledgments: The work was performed in the framework of the Program for Basic Research of State Academies of Sciences for 2013−2020.

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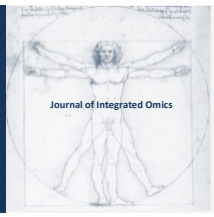

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE 2ND INTERNATIONAL CAPARICA CONFERENCE IN SPLICING (SPLICING 2018)

Regulation of coagulation by alternative splicing

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ABSTRACT

The coagulation system is responsible for the formation of fibrin clots via the integrated and tightly regulated activity of numerous liver-derived plasma proteins. Disruption of the delicate balance between pro- and anticoagulant factors is a major determinant of thrombotic and bleeding disorders. Recent studies have shown that low-abundance splicing isoforms of certain coagulation factors have unique functional properties and play important roles in the maintenance of the haemostatic balance. For example, the fibrinogen γ' chain (4-8% of all fibrinogen γ chains) acts as a thrombin inhibitor and its relative expression is inversely correlated with the risk of venous thrombosis. Similarly, the recently discovered factor V-short isoform (~5% of all plasma factor V) binds the anticoagulant protein tissue factor pathway inhibitor α (TFPIα) with high affinity, resulting in several anticoagulant effects and causing bleeding when up-regulated. Since alternative splicing can be modulated using specific antisense molecules, these natural splicing isoforms represent potential therapeutic targets for the prevention and treatment of coagulation disorders.

Acknowledgments: The work was performed in the framework of the Program for Basic Research of State Academies of Sciences for 2013–2020Financial support by the Dutch Thrombosis Foundation (grants 2011-2 and 2014-1) is gratefully acknowledged.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE 2ND INTERNATIONAL CAPARICA CONFERENCE IN SPLICING (SPLICING 2018)

Human Craniofacial Spliceosomopathies

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ABSTRACT

Germline mutation of core components of the spliceosomes have been associated with range of clinical disorders from isolated retinitis pigmentosa (RP) to developmental syndromes, while somatic mutations of spliceosome components are linked to cell cycle dysregulation and cancer. Recently a group of craniofacial and skeletal disorders caused by dominant mutations in spliceosomal genes has emerged. The first of these to be identified is mandibulofacial dysostosis type Guion-Almeida (MFDGA), caused by haploinsufficiency of the U5 snRNP component EFTUD2. We recently identified haploinsufficiency of SF3B4 as the cause of Nager syndrome, an acrofacial dysostosis (AFD)[1]. Mutation of SF3B4 was subsequently reported in AFD Rodriquez type. SF3B4 is part of the U2 snRNP of the major spliceosome. We also identified heterozygous mutations in a highly conserved regulatory exon of SNRPB as the cause of cerebro-costo-mandibular syndrome (CCMS) [2]. Our experimental data show that these mutations disturb highly conserved exonic splicing silencer sequences crucial to the regulation of the gene's expression. SNRPB encodes a component of the Sm complex of the spliceosome. Additional craniofacial spliceosomopathies result from mutations in splicing factors TXNL4A, RBM8A and CWC27 [3]. The frequent involvement of craniofacial structures invite questions on the sensitivity of craniofacial and in particular pharyngeal arch development to spliceosomal defects, and on the role of the spliceosome in the regulation of development. The report of a young patient with Nager syndrome with bilateral synchronous breast cancer raises the possibility of a link between germline mutation of splicing factors and cancer predisposition.

Many of the craniofacial spliceosomopthies share overlapping features with Treacher Collins syndrome (TCS), a ribosomopathy. Using 3D cranial morphometry, facial morphs (Figure 1) of Treacher-Collins Syndrome (TCS) (N=26) and Nager syndrome (N=13) were classified against 87 craniofacial syndromes, yielding correct classification of TCS and Nager cases of 94% and 90% respectively. These data suggest subtle but distinct differences between the craniofacial features of these syndromes. We propose a comprehensive review of the clinical and molecular features of this emerging group of disorders with a particular emphasis on the role of SF3B4, EFTUD2 and SNRPB in disorders of craniofacial and skeletal development

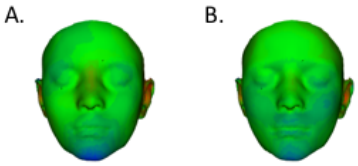

Figure 1. Heat mapped average facial morphs for Treacher Collins (A) and Nager (B) syndromes.

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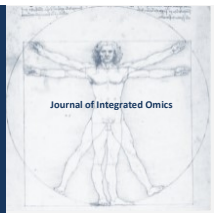

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Towards understanding non-canonical 5' splice site recognition

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Abstract

Most human pathogenic mutations in 5' splice sites affect the canonical GT in positions +1 and +2, leading to non-canonical dinucleotides. On the other hand, non-canonical dinucleotides are observed under physiological conditions in ≈1% of all human 5'ss. It is therefore a challenging task for the understanding of pathogenic mutation mechanisms to examine under which conditions non-canonical 5'ss are used.

To better understand non-canonical 5'ss usage we systematically examined non-canonical 5' splice site selection, both experimentally using splicing competition reporters, and analyzing a large RNA-Seq dataset of 54 fibroblast samples from 27 subjects containing a total of 2.4 billion gapped reads covering 269,375 exon junctions. From both approaches, we consistently derived a non-canonical 5'ss usage ranking

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Molecular insights into the splicing regulation of the *DMD* gene

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ABSTRACT

The splicing regulation of the huge Duchenne Muscular Dystrophy (DMD) gene containing 79 exons remains largely unknown [1]. With the aim to identify the splicing factors (SFs) that regulate the physiological splicing of the pre-mRNA, a series of splicing factors were depleted from a human muscular cell line (C25cl48) by siRNA treatment and the splicing modulations were analyzed by targeted RNA-seq of the DMD transcript. The silencing of 14 specific factors out of the 16 tested influenced the recognition of at least one exon (increase or decrease). Altogether, 8 different exons were found to be modulated.

We are currently exploring the molecular mechanisms that govern the splicing of these exons in order to better delineate the role of the identified trans-acting splicing factors. To this aim, we combine *in silico* analysis, functional experiments and binding assays [2,3]. We looked for putative cis-acting regulatory sequences involved in the recruitment of the splicing factors by using various bioinformatic tools. We compiled the results from the predictions of RNA binding sites for the selected SFs (RBPmap, ESEFinder, SpliceAid2), the analysis of RNA secondary structure (RNAfold) and sequence/structure conservation data (PhyloP conservation UCSC Track, EvoFold). For the exons of interest, we constructed splicing reporter minigenes. To evaluate the relevance of the predictions, mutations were introduced in the minigenes to abolish putative RNA binding sites or to alter the stability of the secondary RNA structure and we looked at the impact on the inclusion of the exon by RT-PCR. The minigenes were also used to perform functional experiments (depletion and/or overexpression of selected SFs). The most significant findings are presented.

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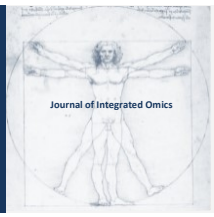

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Development of an antisense-mediated exon skipping therapeutic strategy to correct a frequent causing mutation in Mucolipidosis II

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ABSTRACT

Lysosomal Storage Disorders (LSDs) are a group of rare inherited metabolic diseases caused by the malfunctionb of the lysosomal system, resulting in the accumulation of undegraded substrates inside the lysosomes and leading to severe and progressive pathology. Among them is Mucolipidosis type II (ML II), one of most severe LSDs, which is caused by the total or near total deficiency of the GlcNAc-phosphotransferase, a key enzyme for the correct trafficking of lysosomal hydrolases to the lysosome. GlcNAc-phosphotransferase is a multimeric enzyme and is encoded by two genes: *GNPTAB* and *GNPTG*. One of the most frequent ML II causal mutations is a dinucleotide deletion on exon 19 of the *GNPTAB* gene that disrupts the reading frame and prevents the production of an active GlcNAc-phosphotransferase, which in turn impairs the proper targeting of lysosomal enzymes. Despite broad understanding of the molecular causes behind this and other LSDs, the same progress has not been observed in the development of new therapies, with current treatments still mostly symptomatic and presenting several limitations. Therefore, alternative options should be investigated in order to provide patients and families with better healthcare and more promising therapies. One possibility is the modulation of splicing by antisense oligonucleotides (AOs) with the purpose of altering the splicing pattern, the mature mRNA and ultimately the final protein product. Acknowledging this, the present study intends to design and develop a RNA-based therapeutic agent through the use of AOs capable of inducing the skipping of exon 19 of the *GNPTAB* gene and consequently circumvent the effects of the most common ML II causal mutation. The approach is presently ongoing and different 2'O-Methyl AOs were designed and synthesized to target the *GNPTAB* exon 19 and promote its skipping. We have already succeeded in inducing the skipping of exon 19 in control and ML II patient fibroblasts. The effectiveness of the therapeutic approach regarding the restoration of GlcNAc-phosphotransferase activity was firstly assessed indirectly through the quantification of some lysosomal enzymes in cells. In patient fibroblasts, 48 hours following transfection of the AO which produced the best results, enzyme activity suffered a small increase for all enzymes tested, even if the results are still much lower than those observed for the healthy controls. Currently, experiments are being done to evaluate through radioactive direct assays the GlcNAc-phosphotransferase enzyme activity recovery.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE 2ND INTERNATIONAL CAPARICA CONFERENCE IN SPLICING (SPLICING 2018)

HEXplorer based analysis of splicing regulatory elements

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ABSTRACT

Spliceosome assembly is initiated by the duplex formation between the 11-nucleotide long 5' splice donor (SD) at the exon–intron border and the spliceosomal U1 snRNA, followed by binding of the U2 snRNP to the branch point sequence of the downstream 3' splice acceptor site (SA). The utilization of a splice site within a pre-mRNA is mainly dependent on the intrinsic strength of its SD and SA, which can be scored using algorithms such as MaxEnt score or HBond score. Splice donor choice, however, is not only directed by the spliceosome, but crucially depends on RNA binding proteins recognizing splicing regulatory elements (SREs) in the vicinity of splice sites. Two protein families, SR (serine-arginine-rich) and hnRNP (heterogeneous nuclear ribonucleoparticle) proteins are involved in the position dependent regulation of splice site choice. In particular, SR proteins enhance SD usage when bound to the upstream exon, while they repress splicing from a downstream position; hnRNP proteins affect SD usage the other way round [1].

Cryptic sites are generally splicing inactive as long as the authentic SD is functional; however, if the authentic splice site is weakened by mutations, the splicing outcome can be unclear. It is estimated that roughly 10% of human inherited diseases arise from mutations that affect splice sites directly while another 15% are due to alterations of SREs in the vicinity. Therefore, computational identification of aberrant splice donor usage due to nucleotide changes in or nearby splice sites is in high demand, since this evaluation might be crucial for the clinical treatment of patients [2].

An *in silico* bioinformatic tool, the HEXplorer algorithm, aims to accomplish this daunting task (https://www2.hhu.de/rna/html/hexplorer_score.php) [3]. It was developed with a RESCUE-type (Relative Enhancer and Silencer Classification by Unanimous Enrichment) approach based on a dataset of 43,464 constitutively spliced canonical annotated human exons. Taking overlapping hexamers into account, HEXplorer score profiles reflect potential enhancing or silencing properties of regions in the vicinity of splice sites and also allow the evaluation of changes due to mutations.

To study HEXplorer prediction accuracy with respect to splice site choice, a well-described pathogenic intronic mutation downstream of EαPDH exon 7 was analysed. This mutation has been shown to activate a cryptic splice site in the intron with an even higher U1 snRNA complementarity by creating a de novo SR binding site as indicated by its HEXplorer profile. Furthermore, a randomly selected exon with an unused upstream cryptic splice site of comparable complementarity as the physiological SD was analysed by HEXplorer-guided mutagenesis predicting decreased enhancing property of the region between the cryptic splice site and the constitutive splice donor.

Together with various other results it can be demonstrated that the HEXplorer is a powerful tool to predict SREs and the splicing outcome and might be suitable as diagnostic tool in a clinical context .

Acknowledgments: Funding was provided by Jürgen Manchot Stiftung Molecules of Infection (MOI III) Graduate School.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE 2ND INTERNATIONAL CAPARICA CONFERENCE IN SPLICING (SPLICING 2018)

Alternative Splicing of Four Opioid Receptor Genes Family Suggests an Existence of New Conserved Functional Receptor Isoforms with Unique Structure of mu Opiod Receptor MOR

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ABSTRACT

The signaling of endogenous opioid peptides and opioid drugs is conveyed by the four members of the opioid receptor family: the mu-, delta- and kappa-opioid receptors and nociceptin receptor ((MOR, DOR, KOR, and NOP/ORL1). They participate in the regulation of a number of complex functions such as pain processing, emotions and reward, and immune responses. Canonical opioid receptors are 7-transmembrane spanning (7TM) G-protein coupled receptors (GPCRs), which upon agonist stimulation inhibit adenylate cyclase and the formation of cAMP (mainly Gαi-mediated), open the inwardly rectifying potassium channels and attenuate calcium currents to inhibit cellular excitation. All opioid receptors are coded by single multi-exonic genes, namely *OPRD1, OPRM1, OPRK1* and *OPRL1*. The four genes have evolved from a single ancestral opioid receptor gene through two rounds of genome duplication, with an intermediate stage of MOR/DOR and KOR/NOP ancestors [1]. Alternative splicing presents an intriguing determinant in opioid receptors diversity because it leads to profound structural alterations of the receptor, which in turn change ligand selectivity, trafficking of the receptor, and receptor signaling, in some cases to the opposite ones. We applied 5'RACE PCR to discover previously unknown exons or splicing sites in all four opioid receptors. Our approach identified a number of novel alternative functional transcripts in addition to the main transcript. In particular, *OPRM1* has at least 19 exons shuffled into over 30 transcripts in human. These transcripts code for three types of receptor isoforms: full-length 7TM receptors with varying C-termini, N-terminally truncated 6TM receptors, and highly truncated N-terminal 1TM fragments, which all have distinct characteristics and functions [2]. The transcriptional landscape of other opioid differs strikingly from *OPRM1* as they present only few alternative transcripts though they are coding for the same types of receptor isoform. It is intriguing why the *OPRM1* gene codes for so many more alternatively spliced forms than three other highly related opioid receptors, and how these truncated receptors isoforms within opioid receptor family are related to other GPCRs family members .

Acknowledgments: Funding for this work is supported through the Canadian Excellence Research Chairs (CERC) Program (grant CERC09 to L.D.).

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE 2ND INTERNATIONAL CAPARICA CONFERENCE IN SPLICING (SPLICING 2018)

Scaf6 (DmCHERP) regulates muscle development and function in *Drosophila*

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ABSTRACT

Muscles are essential for animal survival, with functions as diverse as pumping blood, allowing movement and facilitating digestion. Muscles move by contracting, a process dependent on Ca^{2+} release from internal muscle stores. There is a complex machinery that regulates this process, including potentially the recently identified gene CHERP (Calcium Homeostasis Endoplasmic Reticulum Protein). CHERP was identified to bind Ryanodine Receptor 1 in the sarcoplasmic reticulum of rat skeletal muscle [1], directly linking it to Ca^{2+} regulation, but more recent reports suggest CHERP is actually a nuclear-localized protein which functions in mRNA-splicing [2,3]. To unequivocally establish an in vivo functional role for CHERP in muscle and to investigate its molecular mechanism, we are studying the *Drosophila* homolog, SR-related CTD associated factor 6 (Scaf6). Scaf6 was identified to regulate the trafficking of *gurken* mRNA in *Drosophila* ovary [4], supporting its function in mRNA binding. Here we confirm by mRNA-Seq and RT-PCR that Scaf6 is expressed in adult muscles. We characterize the Scaf6 localization pattern in muscle using an endogenous epitope tag. We confirm that Scaf6 plays an essential functional role in muscle, as muscle-specific RNAi as well as whole-animal mutants are flightless and display a variety of other behavioral defects. We show that the flight defect arises developmentally, as the flight muscle fibers are detached and atrophic from 48 h after puparium formation. We have further investigated the mechanism of Scaf6 function using mRNA-Seq at two timepoints during flight muscle development. Our results demonstrate a function for Scaf6 in muscle, highlighting the importance of alternative splicing during myogenesis and suggesting a similar role for CHERP in vertebrate muscles .

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE 2ND INTERNATIONAL CAPARICA CONFERENCE IN SPLICING (SPLICING 2018)

Understand molecular mechanisms of *NXNL1* gene's alternative splicing should be a benefit therapeutic treatment of RP

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ABSTRACT

Most forms of retinitis pigmentosa (RP) lead to a primary rod photoreceptor death responsible to night vision. This cells degeneration is followed by secondary cone death necessary for visual acuity leading to patient blindness. The bifunctional gene *NXNL1* encodes two proteins by an alternative splicing, the short *NXNL1* messenger encodes RdCVF that is secreted by rods and protects cones and the long *NXNL1* messenger the enzyme RdCVFL. The mode of action and the signalization of RdCVF and RdCVFL have been identified. RdCVF stimulates the renewal of cones outer segments through the membranal BSG1/GLUT1 complex and RdCVFL protects the cones against oxidative damages [1,2]. Cones of mouse retina express only one of the two NXNL1 gene products, the thioredoxin RdCVFL [3]. This is also shown in primate retina (Fig.1). The alternative splicing leading to intron retention that results in the production of the RdCVF mRNA from the *NXNL1* gene is taking place only in rods. We have identified a conserved stem loop renamed NXNL1 responsive element (NRE) in the *NXNL1* pre-mRNA that is very likely involved in intron retention which can bind several proteins (Fig.2). Mimic molecular mechanisms leading to the *NXNL1* gene's alternative splicing in cone as rods normally do should lead to RdCVF expression and secretion for encourage cone survival in an autocrine manner through the BSG1/GLUT1 complex.

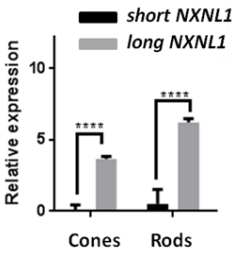

Figure 1. Only rods express both messengers of NXNL1 gene.

Figure 2. NRE secondary structure.

Acknowledgments: Valerie Forster for helping on human and macaca retina dissection and Molecular Imaging Research Center (MIRCen) that provided us macaca's eye-ball.

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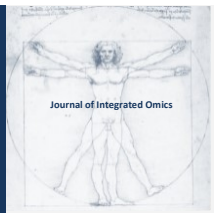

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE 2ND INTERNATIONAL CAPARICA CONFERENCE IN SPLICING (SPLICING 2018)

The effects of metabolic conditions on alternative splicing

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Abstract

Type 2 diabetes is a metabolic disorder associated with chronic hyperglycaemia and hyperlipidaemia, with consequent insulin resistance and a progressive deterioration of pancreatic beta cell mass and function. The combined deleterious effects of these metabolic changes on beta cells are known as glucolipotoxicity (GLT) [1]. Alternative splicing is a post-transcriptional process that allows a single gene to encode multiple transcripts and is a crucial mechanism for generating proteomic diversity [2]. However, little is known about the role of alternative splicing in diabetes. The aim of this study was to test whether metabolic conditions relevant to type 2 diabetes affect alternative splicing in beta cells.

INS-1 beta cells were treated under GLT or control conditions and the expression of a panel of genes important for the regulation of alternative splicing was examined by quantitative reverse-transcription PCR (qRT-PCR). These experiments showed that GLT conditions significantly increase expression of the splicing regulator *Elavl1* in beta cells ($p=0.04$, $n=4$ independent experiments). The role of *Elavl1* in beta cells is not known, but it has been shown previously that siRNA knockdown of the related family member, *Elavl4*, increases beta cell apoptosis [3], suggesting that this family of genes regulates beta cell survival. Using a combination of exon arrays and a candidate gene approach, we have further shown that the increased *Elavl1* expression in beta cells following GLT treatment is associated with altered exon splicing of key beta cell genes, as well as changes in gene expression.

This study demonstrates that metabolic factors increase gene expression of the alternative splicing regulator *Elavl1* in beta cells and induce changes in the splicing of beta cell genes. This suggests that the metabolic conditions existing in many people with type 2 diabetes may cause aberrant alternative splicing in beta cells

Acknowledgments: Valerie Forster for helping on human and macaca retina dissection and Molecular Imaging Research Center (MIRCen) that provided us macaca's eye-ball.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE 2ND INTERNATIONAL CAPARICA CONFERENCE IN SPLICING (SPLICING 2018)

Control of mRNA splicing by noncoding intragenic RNA elements that evoke a cellular stress response

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ABSTRACT

Once activated by double helical RNA, mammalian RNA-dependent protein kinase, PKR, will phosphorylate its substrate, translation initiation factor eIF2α, to inhibit protein synthesis. eIF2α phosphorylation is critical for mounting a cellular stress response. We have discovered within cellular genes short, 100-200 nucleotide long elements that, once transcribed, form RNA structures that potently activate PKR in the vicinity of the RNA and thereby strongly regulate gene expression. Intragenic RNA activators of PKR can (*a*) attenuate translation of the gene by activating PKR and thereby inducing eIF2α phosphorylation (example: *IFN-γ* gene [1]), or (*b*) greatly enhance nuclear splicing efficiency by activating PKR and inducing eIF2α phosphorylation, thereby promoting early spliceosome assembly (examples: *TNF-α* [2], adult and fetal *globin* genes [3]) - thus considerably extending the potential scope of gene regulation by these elements. The *TNF-α* RNA activator of PKR (2-APRE) folds into a pseudoknot conserved from teleost fish to humans, critical for PKR activation and mRNA splicing, to link stress signaling to protective immunity. Excision of *β-globin* first intron induces strand displacement within the PKR activator, a structural rearrangement that silences the ability of spliced *β-globin* mRNA to activate PKR. Thus, the ability to activate PKR remains transient, serving solely to enable splicing.

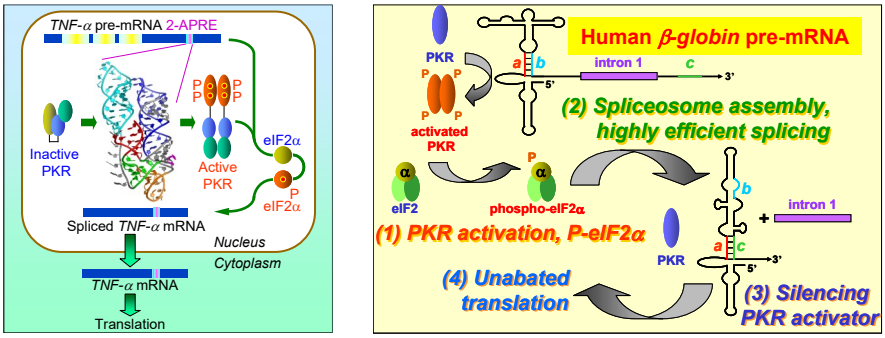

Figure 1. RNA activators of PKR in *TNF-α* and *β-globin* pre-mRNA upregulate splicing via eIF2α phosphorylation.

Acknowledgments: Supported by grants from the Israel Science Foundation and the German Research Foundation (DFG).

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE 2ND INTERNATIONAL CAPARICA CONFERENCE IN SPLICING (SPLICING 2018)

Aberrant binding of RNA processing proteins to a novel macrosatellite-derived lncRNA in colorectal cancer

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ABSTRACT

We have identified a pericentromeric macrosatellite undergoing severe demethylation in a subset of colorectal tumors [1]. When demethylation is accompanied by histone acetylation, it leads to transcription of this macrosatellite repeat into lncRNAs that preferentially localize as perinucleolar aggregates and interact with SAM68 nuclear bodies. We have globally named these novel ncRNAs as *TNBL*, for Tumor-associated NBL2 transcript [2]. Moreover, *TNBL* binds several proteins involved in nuclear functions and RNA metabolism, such as CELF1 and NPM1. Global DNA hypomethylation in cancer occurs mainly in the repetitive fraction of the genome, and it has been associated to chromosomal aberrations. However, the causal mechanisms linking demethylation of repeats with genomic instability are not clear. Whether this lncRNA could bridge both processes, have an impact on splicing regulation or merely mark a subpopulation of tumor cells with distinct vulnerabilities is under investigation.

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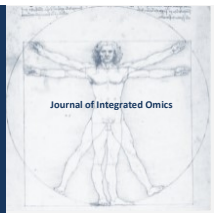

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RNA in Genomic Medicine

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ABSTRACT

One in seventeen people are born with or develop a rare disease during their lifetime. 1 in 3 will get cancer. At least 80% of rare diseases have an identified genetic component, with 50% of new cases of rare diseases being identified in children. Altogether a significant health burden. Accurate diagnosis is fundamental in medicine providing answers and facilitating informed decision-making for families and their doctors particularly with regards to personalised medicine.

Despite the success of genome sequencing, the current diagnostic rate for genomic analyses across a variety of rare diseases is 25 -40%. We are investigating how this can be significantly improved if we consider splicing and undertake RNA sequencing (RNA Seq) or transcriptomics as a complementary diagnostic tool.

With > 10 million sequence variants seen between one individual and another of unknown clinical significance on sequencing, deciding which are pathogenic is a mammoth task. We also know that sequence changes can affect the complex splicing process which is much harder to incorporate into genomic analyses.

I will present examples of disease caused by disruption of the splicing process from our own lab. as well as other significant published examples as supporting evidence for the importance of developing this arm of diagnostic practice. Other forms of RNA, for example non-coding RNA, have also been shown to be important in disease and should be incorporated in a diagnostic pathway.

In association with the NIHR and Genomics England. We have begun to develop diagnostic pathways to take this into account, both by developing bioinformatics and high throughput transcriptome methods for assessing splicing abnormalities. In this way we will expand our capabilities and find 'missing' mutations alongside potential biomarkers and enable future potential precision medicine.

Keywords: Splicing, transcriptomics, RNA.

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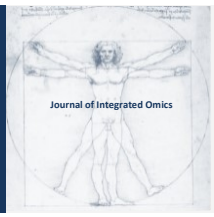

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE 2ND INTERNATIONAL CAPARICA CONFERENCE IN SPLICING (SPLICING 2018)

Chimeric RNAs Generated By Intergenic Splicing And Their Implications

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Abstract

The completion of Human Genome Project is only the end-of-the-beginning. Many traditional views regarding genes and the genome are being challenged with new knowledge gained through efforts such as ENCODE. However, it is still widely believed that genes and gene products (RNAs and proteins) are not meant to intermingle except in pathological situations, i.e., cancer. Interestingly, RNA-sequencing analyses from several non-cancer sources resulted in identifying populations of chimeric fusion transcripts, raising the possibility that fusion RNAs and proteins are a rather widespread phenomenon. Our work on RNA trans -splicing and intergenic cis-splicing have helped open a new paradigm for RNA level processes that generate functional fusion products, and provides evidence that the intergenic splicings may be a mechanism to expand functional genome. On the other hand, misregulation of these chimeric RNAs may contribute to diseases including cancer, diabetes and neurodegenerative diseases.

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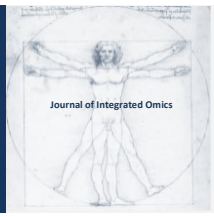

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE 2ND INTERNATIONAL CAPARICA CONFERENCE IN SPLICING (SPLICING 2018)

Massive upregulation of alternative/aberrant splicing by small molecules that target SF3B1

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Abstract

The spliceosome has recently been identified as an attractive target for the development of anticancer agents, with most compounds acting against SF3B1. Molecules that have been identified that interact with this protein include spliceostatin A, meamycin, sudemycin and herboxidiene. Interestingly, these compounds demonstrate considerable selectivity for tumor cells with significantly lower IC50 values for growth inhibition as compared to normal cells. The reason for this is unclear and hence considerable effort has been targeted towards identifying the molecular consequences of SF3B1 inhibition in tumor cells.

Initial experiments indicated that agents that interacted with the spliceosome resulted in a massive upregulation of alternative splicing (AS), with the generation of novel transcripts that were not present within the standard RNA databases. Therefore, we developed a new bioinformatics analysis pipeline that analyzed the sequences adjacent to the exon junctions to identify and quantitate the aberrant RNAs that were expressed in cells exposed to these molecules. Using this approach, coupled with quantitative RT/PCR, we confirmed the presence and levels of these novel RNAs. These results verified the effectiveness and ability of the computational approaches to analyze transcripts from genes that were expressed at both high and low levels, and allowed us to document the numerous, as yet unreported, RNA isoforms that arose via AS.

Consistent with previous findings, in RNAseq data obtained from tumor cells exposed to compounds that inhibit SF3B1, massive upregulation of AS was detectable. These changes in splicing were readily detected by molecular approaches. Interestingly however, we saw pleiotropic effects depending upon which agent was used. For example, when cells were exposed to sudemycin D1 or herboxidiene, the cohort of genes that were subject to AS were similar. However, when spliceostatin A was used, a different cadre of changes was seen. Overall, our results suggest that compounds that target SF3B1 do not induce common molecular AS events, even in the same cell type. Therefore, the development of our RNA analysis pipeline has allowed us to identify and understand both subtle and global effects with respect to AS, and will allow a more detailed evaluation of the biological consequences of exposure of cells to spliceosome-targeting agents.

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Uveal Melanomas with SF3B1 Mutations: A Distinct Subclass Associated with Late-Onset Metastases

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ABSTRACT

Uveal melanoma(UM) patients can be stratified on basis of recurrent gene mutations, copy number variations (CNV) or gene expression patterns. Half of the UM patients display monosomy of chromosome 3 and the remaining allelic copy of the BAP1 (BRCA-associated protein 1) protein is inactivated by gene mutation leading to early metastatic disease. Somatic mutations in SF3B1 (splicing factor 3, subunit 1) and *EIF1AX* (eukaryotic initiation factor 1A, X-linked) are almost exclusively observed in disomy 3 tumors. The N-terminal part of *EIF1AX* is changed by an in frame mutation of the first 15-20 amino acids of this protein in 20% of UM patients, and these patients will seldom develop metastasis. *SF3B1* mutations seen in 20-25% of UM patients are with a few exceptions targeted at codon p.R625. UM patients with a somatic *SF3B1* mutation are in general younger and appear to have a favorable prognosis compared to patients with a BAP1 mutation but in our longer follow-up study it was shown that eventually also these patients will develop metastases[1]. Using SNP array data of 214 UM patients and/or conventional karyotyping (n=119) we investigated a possible relationship between mutational status of *BAP1, SF3B1*, and *EIF1AX* uveal melanoma and CNV patterns. We showed that BAP1-negative UMs have the largest CNVs in size and *SF3B1*-mutated UMs harbored the most CNV events. Whereas *EIF1AX*-mutated UMs were characterized by the lack of CNVs. Iso-chromosomes were seen almost exclusively in BAP1 negative UMs. Mutations in these genes are strongly associated with distinct molecular subclasses[2]. This highlights and reflects the biological difference between UMs on a genetic level.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE 2ND INTERNATIONAL CAPARICA CONFERENCE IN SPLICING (SPLICING 2018)

Splicing mutations in neurometabolic diseases: mechanisms and therapies

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ABSTRACT

Our group works in the field of inherited metabolic diseases, genetic defects that affect a metabolic pathway or cellular process and commonly characterized by a specifically altered biochemical profile that guides the diagnosis. Splicing mutations account for 10-15% of the mutant alleles in the diseases that we have characterized genetically, mainly organic acidemias and amino acid disorders. We have detected several deep intronic mutations resulting in aberrant pseudoexon insertion and exonic mutations that create or activate cryptic splice sites. Targeted therapy using splice switching oligonucleotides (SSOs) have been used with success to correct these splicing defects, resulting in near-normal levels of transcript, protein and enzymatic activity in patients' cells. We have also explored the therapeutical potential of adapted U1 snRNAs fully complementary to mutated 5' splice sites to correct 5' splice mutations.

Our most recent work in collaboration with Dr B.S. Andresen (University of Southern Denmark) involves the characterization of the pathogenic effect of variants c.1199+17g>a and c.1199+20g>c, identified in the PAH gene in patients with phenylketonuria. Both mutations cause exon 11 skipping in a minigene system and RNA binding assays indicate that binding of U1snRNP70 to this region is disrupted, concomitant with a slightly increased binding of inhibitors hnRNPA1/H. We have performed deletion and point mutagenesis and overexpression of adapted U1snRNP to identify critical motifs involved in the regulation of correct splicing at the natural 5' splice site. The results indicate that U1snRNP binding at the intronic region is determinant for efficient exon 11 splicing. In this work, we expand the functional effects of non-canonical intronic U1 snRNP binding by showing that it may enhance exon definition and that, consequently, intronic mutations may cause exon skipping by a novel mechanism, where they disrupt stimulatory U1 snRNP binding close to the 5' splice site. Notably, our results provide further understanding of the reported therapeutic effect of exon specific U1 snRNA for splicing mutations in disease.

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Simultaneous multi-sample analysis significantly improves alternative splicing detection

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ABSTRACT

Alternative splicing is a key gene regulatory mechanism with important roles in development and disease. Transcript assembly from RNA-seq reads and diffferential splicing (DS) analysis are two key problems that occur in all transcriptomics applications, but despite significant efforts to design adequate analysis tools, both transcript reconstruction and DS detection are not meeting their full potential. Parting with the traditional one-at-a-time approach to analyzing RNA-seq samples, we developed two tools that simultaneously analyze all samples in an RNA-seq collection to produce significantly improved results. CLASS3 is a novel transcript assembler that uses a splice graph model to combine features from multiple samples into a unified data structure, which increases accuracy and consistency. JULiP++ selects a reliable set of introns from a set of RNA-seq samples using an L1-regularized program and detects introns that are differentially present between groups of samples. CLASS3 compares favorably to the state-of-the-art in transcript assembly, including assemblers StringTie and Scallop, whereas JULiP++ outperforms existing differential splicing tools, including rMATS, Junction-seq and the recently published LeafCutter. Both tools can be downloaded from our web site: http://florealab.org.

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