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The \textit{NBPF} Gene Family

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1. Introduction

Neuroblastoma is one of the most intensely studied solid malignancies that affect children (Maris & Matthay, 1999). These tumours are heterogeneous biologically and clinically. One subset of neuroblastoma is susceptible to spontaneous apoptosis with little or no therapy and another subset differentiates over time, but most of these tumours are difficult to cure with current treatments. A relatively high proportion of affected children die due to resistance of the neuroblastoma to therapy (Van Roy et al., 2009). Every step in the identification or functional understanding of the genes that play important roles in this cancer could bring us closer to understanding the molecular mechanism and could help to develop more effective therapy.

We will describe the novel \textit{NBPF} gene family and its role in neuroblastoma. The \textit{NBPF} gene family was originally identified by the disruption of one of its members in a neuroblastoma patient (Vandepoele et al., 2008). This gene was named \textit{NBPF1}, for Neuroblastoma Breakpoint Family, member 1. Several reports indicate that the \textit{NBPF} gene family might play an important role in neuroblastoma and possibly in other cancers as well. Additionally, this evolutionarily recent gene family has been involved as an important player in human evolution.

2. Identification of \textit{NBPF1} during the positional cloning of a constitutional translocation in a neuroblastoma patient

In 1983, karyotypic analysis of fibroblasts from a Belgian neuroblastoma patient revealed a \textit{de novo}, constitutional translocation between chromosomes 1p36.2 and 17q11.2 (Laureys et al., 1990; Laureys et al., 1995). These two chromosomal regions are often rearranged in sporadic neuroblastoma tumours (Savelyeva et al., 1994). So it was believed that the translocation had predisposed the patient to neuroblastoma by disrupting one or more genes. Although previous cytogenetic experiments indicated that the translocation was balanced, analysis of the breakpoint sequences showed greater complexity (Figure 1). First, in both derivative chromosomes, a few nucleotides of unknown origin were inserted between the chromosome 1 and 17 sequences (Vandepoele et al., 2008). Such insertions are frequently observed in reciprocal constitutional translocations (Willet-Brozick et al., 2001). Second, on chromosome 1, the translocation resulted in the deletion of a genomic fragment of 5,215 base pairs that is present in the human genome reference sequence (Vandepoele et
However, numerous reports describe structural variation at this locus (see below), which makes it impossible to determine the precise size of the deletion in this neuroblastoma patient. No deletions or insertions of genomic regions were observed on chromosome 17, which means that the translocation at this level is indeed balanced. Notably, both translocation breakpoints are located in LINE repeats, classified as type L1-PA4 for chromosome 1 and as type L2 for chromosome 17, but no significant sequence homology between these two LINE repeats was found. Therefore, most probably these repeats do not participate in the mechanism of this translocation (Vandepoele et al., 2008).

Fig. 1. Genomic overview of the translocation breakpoint of the der(1) and der(17) chromosomes.

**Central box:** Normal 1p36 and 17q12 chromosomes. Both translocation breakpoints (BP) on chromosome 1 are located in intronic regions of the NBPF1 gene, and the translocation results in deletion of 5,215 bp. The translocation breakpoint on chromosome 17 is located in the ACCN1 gene and disrupts only isoform 2. **Top:** On the der(1) chromosome, the translocation gives rise to two chimeric transcripts of NBPF1 sequences (NBPF1-CHI) fused to sequences derived from chromosome 17 (grey box), thereby extending the open reading frame. Translocation of the promoter and the first exon of ACCN1 isoform 2 might result in chimeric transcripts on der(1), but so far no transcripts have been isolated (question mark with dotted line). **Bottom:** On the der(17) chromosome, isoform 2 of the ACCN1 gene is probably no longer expressed due to loss of its promoter and first exon (dotted line with question mark), whereas the last three NBPF1 exons are translocated to the der(17) chromosome (exon types 11, 12 and 14) (Modified after Vandepoele et al., 2008).
On chromosome 17, the translocation disrupts ACCN1, a potential glioma tumour suppressor gene (Vila-Carriles et al., 2006). ACCN1 encodes a member of the superfamily of amiloride-sensitive degenerin/epithelial sodium channels (DEG/ENaC) and is also known as MDEG (Waldmann et al., 1996), BNaCl (Garcia-Anoveros et al., 1997) and ASIC2 (Waldmann et al., 1997). ACCN1 is expressed primarily in the brain, where it might play a role in neurotransmission (Garcia-Anoveros et al., 1997). Alternative initiation of transcription results in two variants encoding distinct isoforms differing in their N-terminal domains (Lingueglia et al., 1997). The translocation in the neuroblastoma patient disrupts only isoform 2 of the ACCN1 gene. Translocation of the promoter and the first exon of ACCN1 isoform 2 could result in chimeric transcripts from the derivative chromosome 1 (der(1)), but no such transcripts could be identified. Isoform 2 is probably not expressed from derivative chromosome 17 (der(17)) due to the loss of its promoter and first exon (Figure 1) (Vandepoele et al., 2008).

Oncomine expression analysis (Rhodes et al., 2007) of ACCN1 showed that it is expressed at significantly lower levels in neuroblastoma tumours with MYCN amplification, in higher stage tumours, and in tumours with 1p36 loss of heterozygosity. These findings indicate that ACCN1 plays a role in tumour aggressiveness (Vandepoele et al., 2008). ACCN1 also has a role in oncogenesis of glioma cells, in which functional restoration of ACCN1 at the plasma membrane decreases cell migration (Vila-Carriles et al., 2006). Gain of chromosome 17q is the most frequent genetic alteration observed in neuroblastoma, but the biological basis is complex because dosage alterations involve genes localised on both sides of the 17q breakpoints. It has been suggested that genes mapping between 17cen and 17q12 suppress tumour progression, whereas genes mapping between 17q23.1 and 17qter promote its progression (Lastowska et al., 2002). These findings further stress the possible role of ACCN1 as a tumour suppressor of neuroblastoma.

Cloning of the chromosome 1 breakpoint identified a novel gene that is disrupted by this translocation. This gene was named NBPF1, for Neuroblastoma Breakpoint Family, member 1. NBPF1 is a member of a newly discovered gene family with an intricate genomic structure (see section 3.1). The translocation truncates NBPF1 and gives rise to two chimeric transcripts of NBPF1 sequences fused to sequences derived from chromosome 17, thereby extending the open reading frame with either 34 or 11 additional codons. The breakpoint of the der(1) chromosome is located in an intron between exon types 7 and 8 (Figure 1). The translocation breakpoint of the der(17) chromosome is also intronic, but it is located between exon types 13 and 11, which results in deletion of a part of the NBPF1 gene, as described above. The three last NBPF1 exons, of type 11, 12 and 14, are translocated to the der(17) chromosome, but so far no chimeric transcripts have been described (Figure 1) (Vandepoele et al., 2008).

We determined the expression level of NBPF1 in 32 neuroblastoma cell lines. This profiling showed that NBPF1 expression varied widely between the different samples, in line with a previous expression analysis of the total NBPF family (Vandepoele et al., 2008). Interestingly, we did not observe complete loss of NBPF1 expression in any cell line, as has been reported for other genes located in the 1p36 region (Janoueix-Lerosey et al., 2004; Fransson et al., 2007). Statistical analysis of these data revealed a significantly lower expression level of NBPF1 in cell lines with a heterozygous deletion of the NBPF1 locus than in cell lines without NBPF1 loss (p<0.05), indicating that NBPF1 could be a tumour suppressor gene (TSG) in neuroblastoma. Although this decreased expression of NBPF1 might be explained...
by the lower gene copy number, it is striking that such downregulation was observed for only 15–20% of the genes affected by hemizygosity of this region (Janoueix-Lerosey et al., 2004). Therefore, in addition to loss of heterozygosity, other mechanisms probably play a role in downregulation of some of these genes. It has been proposed that the combined decrease in the expression of these genes, rather than the inactivation of one single classical TSG, could cause the unfavourable outcome associated with 1p deletions in neuroblastoma (Fransson et al., 2007). Oncomine analysis (Rhodes et al., 2007) showed that in two studies there was no significant difference in NBPF expression between different tumour grades or INSS stages but one study showed decreased NBPF levels in neuroblastoma tumours that relapsed within five years, which also indicates that NBPF1 might suppress the development of neuroblastoma. More importantly, induced NBPF1 expression in human colon cancer cells decreased their clonal growth in a soft agar assay, demonstrating that NBPF1 can act as a TSG, at least in colon cancer (Vandepoele et al., 2008). Like neuroblastoma, colon cancer is characterised by frequent deletions or translocations of 1p36 (Schwab et al., 1996). Indeed several reports indicate that the same TSG might be implicated in both neuroblastoma and colon cancer (Kong et al., 1997; Reyes-Mugica et al., 1998).

3. The NBPF gene family

3.1 Structure of the NBPF gene family

The cloning of the breakpoints of the constitutional translocation in a neuroblastoma patient revealed that this translocation had disrupted NBPF1 (see section 2). This gene is a member of the NBPF gene family, which is located primarily on duplicated regions of chromosome 1 (Figure 2A). In neuroblastoma, these regions frequently contain aberrations. For instance, one third of primary neuroblastoma tumours carry a deletion of the 1p36 region (White et al., 2005). The NBPF genes have a repetitive structure with high intergenic and intragenic sequence similarity, both in coding and non-coding regions (Vinogradova et al., 2002; Vandepoele et al., 2005). The NBPF1 gene consists of 25 coding exons, but on the basis of their high sequence identities, these exons can be classified into 14 different exon types (Figure 2B). All other NBPF genes contain variable numbers of some or all of the exon types present in NBPF1, but some genes also contain exons without significant homology to the NBPF1 exons. The NBPF gene family consists of 24 members located primarily on three regions of chromosome 1, namely, 1p36, 1p12 and 1q21, but two members, NBPF21P and NBPF22P, are located on chromosomes 3p and 5q, respectively.

Several members of this gene family are abundantly expressed in different human cell lines and human tissues, but no discernable orthologs could be found in the genomes of mouse or rat. Nevertheless, the NBPF genes do not appear to be restricted to primates because sequences with low but significant homology to NBPF have been identified in bovine and canine genomes (Vandepoele et al., 2005). We previously analysed the genomic organisation of the NBPF gene family (Vandepoele et al., 2005). For that analysis, we used the reference sequence of the human genome of the NCBI build 35, which is an improvement over the first release because it contains only 341 gaps (IHGSC 2004) instead of the 150,000 gaps that were present in the first draft version. However, the NBPF genes are located in regions that are refractory to all standard sequencing efforts. In this analysis, we obtained numerous small contigs that contained only partial NBPF genes. To provide an up-to-date description of the NBPF genes in the human genome reference sequence, we now report on the analysis of NBPF sequences in the current human genome release (Build 37.2).
Fig. 2. Genomic overview of the NBPF gene family.
Legend to figure 2: Genomic overview of the NBPF gene family. A/ Ideogram of human chromosome 1 showing the localisation of the NBPF genes. The arrow in front of each gene indicates its orientation in the chromosome. The colour of the gene symbol indicates whether it is full-length (green; start and stop codons present in one continuous ORF in the predicted cDNA), partial (red; due to gaps in the genomic sequence), or a pseudogene (black, P). Vertical lines indicate whether genes are present in a single genomic contig of the human genome assembly (NCBI Build 37.2). The two genes not linked to the ideogram, NBPF21P and NBPF22P, are located on other chromosomes. B/ The open reading frames of the different NBPF genes were assembled in silico based on cDNA sequences. NBPF4 to NBPF6 and NBPF22P contain exons with no significant homology to exons present in NBPF1. These are shown as gray boxes with letters instead of numbers. NBPF8 contains a deletion of two nucleotides in the first coding exon, which results in a frameshift mutation. Due to skipping of the type-2 exon in several NBPF8 transcripts, this frameshift often does not result in a premature stop. NBPF-X sequences are new NBPF members that still lack an official gene symbol. Frameshift and nonsense mutations in pseudogenes are indicated by asterisks. Three consecutive dots indicate the presence of gaps in the current genomic assembly (Updated from Vandepoele et al., 2005).

In addition to the 22 NBPF genes we identified before (Vandepoele et al., 2005), two additional NBPF genes are currently present in GenBank: NBPF23 and NBPF24. Copy number variations of NBPF23 were recently shown to be involved in neuroblastoma (see below) (Diskin et al., 2009). In our previous analysis, we only focused on the coding exons, and so the 5'UTR was not properly annotated in the databases. However, the NBPF23 sequences are upstream of the NBPF17P encoding exons at a similar distance as the 5'UTR exons of NBPF1 are from its coding sequences. We therefore believe that the NBPF23 gene is not a novel gene but is the 5'UTR of NBPF17P. We described NBPF17P as a pseudogene because it contains nonsense mutations in the normal coding sequence (Vandepoele et al., 2005). In view of that, it is interesting that Diskin et al. (2009) found that this sequence is strongly expressed in several tissues, which indicates that this “pseudogene” might have a function. The other new NBPF member in GenBank is NBPF24, but this is still a hypothetical sequence predicted from a fragment of genomic DNA (see below).

The short arm of chromosome 1 contains two regions that harbour NBPF genes. The 1p36 locus contains the NBPF1 gene. Additionally, one functional gene (NBPF3) and a partial duplication of this gene (NBPF2P) are located proximal to NBPF1. Closer to the centromere on 1p12, there is a second cluster of NBPF genes (NBPF4 to NBPF7). These genes are very similar to each other and make up a separate branch of the NBPF evolutionary tree (Vandepoele et al., 2005). The sequences of these two clusters were not changed in the most recent human genome reference sequence. The updated version of the human genome reference sequence has undergone numerous changes in chromosome 1q. Overall, it now contains more NBPF sequences than the previously published sequence. The NBPF8 gene was the most proximal NBPF gene in our old analysis, but it is now preceded by a novel NBPF member, which we call NBPF-X1. This is the longest NBPF gene we have observed so far, with a size of 77 kb and a cDNA of 11 kb. As there is still a gap in the genomic sequence on the 5' side of NBPF-X1, the gene and its derived cDNA are presumably even longer. Distal to NBPF-X1, NBPF8 is an aberrant member of the NBPF gene family. Deletion of two nucleotides in its first coding exon causes a frameshift. However, this frameshift can be rescued by an alternative splicing event that excludes the second coding exon from the mature mRNA. NBPF9, NBPF10 and NBPF11 are identical to those in the old analysis. Like
NBPF-X1, NBPF10 contains numerous repeats of the 3' exons. NBPF11 contains a frameshift mutation in one of its last exons, but this gene is nevertheless expressed in a variety of tissues, and hence, we consider it a functional gene. NBPF11 is followed by a second new NBPF gene, NBPF-X2. As the 5' end of this gene is located near a gap in the contig, only a partial sequence is available. In our previous analysis, the sequence of the NBPF12 gene was incomplete, with only nine consecutive exons in an uninterrupted contig. In the current genome sequence, the complete NBPF12 gene is present and contains 77 exons. This is followed by NBPF13P, a pseudogene with numerous nonsense mutations in different exons, and the new NBPF24. Interestingly, NBPF24 is an almost identical copy of NBPF11 and contains the same frameshift mutation in one of its last exons. The following gene, NBFP14, has not changed in the current assembly, but it is followed by the last new NBPF gene, NBPF-X3. Like other new members, NBPF-X3 is also composed of many repetitions of the standard NBPF exon types. The identical NBPF15 and NBPF16 genes are contained in the last contig on chromosome 1q, together with the NBPF17P and NBPF18P pseudogenes. In our previous analysis, NBPF19 and NBPF20 were not positioned on chromosome 1. In the current assembly, these sequences represent different haplotypes, it is possible that NBPF19 and NBPF20 are not true paralogs of the current NBPF genes, but simply variants of other NBPF genes in another haplotype.

In addition to the NBPF sequences on chromosome 1, two other regions in the human genome contain NBPF-like sequences: 3p22.3 (NBPF21P) and 5q14.2 (NBPF22P). These sequences have only limited homology to the NBPF genes on chromosome 1 but share a common origin and are included in the NBPF family.

Analysis of the predicted protein sequences showed that several pairs of exon types encode a novel protein domain, called the NBPF repeat. The NBPF repeat comprises a PFAM protein domain of unknown function, called DUF1220 (Vandepoele et al., 2005), and we will refer to this repeat as the NBPF/DUF1220 repeat. It is always built of two exons and is present in multiple copies in the different NBPF proteins. It is also present as a single copy with lower homology in the human PDE4DIP gene encoding myomegalin (Figure 3) (Verde et al., 2001). Myomegalin is a centrosomal protein and contains, besides its NBPF/DUF1220 domain, some regions that show homology to CDK5RAP2, a gene that lacks NBPF/DUF1220 sequences. Mutation of CDK5RAP2 has been implicated in microcephaly (Bond & Woods, 2006). There is recent evidence for involvement of the NBPF/DUF1220 repeat in human evolutionary adaptation, cognitive function, and disease, including neuroblastoma (see below). Other than the novel NBPF/DUF1220 repeat, there are no known protein domains in the NBPF proteins.

3.2 The NBPF gene family and human evolution

Several regions on chromosome 1 consist of recently duplicated sequences, and the NBPF gene family is located primarily on such segmental duplications (Vandepoele et al., 2005). Segmental duplications, also known as low copy repeats, are duplicated blocks of genomic DNA longer than 1 kbp and with high sequence identity, and they typically map to two or more locations in the genome (Marques-Bonet et al., 2009b). The number of base pairs mapping to high-identity duplications are similar in the human and mouse genomes, but the segmental duplications in mouse are tandemly organised, whereas in humans most of them are interspersed (She et al., 2008). Additionally, human segmental duplications tend to
Fig. 3. Exon organisation of the NBPF1 gene and the predicted domain structure of the translated NBPF1 protein.

A/ Analysis of the predicted NBPF protein sequences showed that each of several pairs of exon types encodes a novel protein domain, called the NBPF repeat. The NBPF repeat comprises a PFAM protein domain of unknown function, called DUF1220 (see C). It is always built of two exons and is present in multiple copies in the different NBPF proteins. Three coiled-coil regions were predicted in the N-terminal domain of the NBPF1 protein. Each coiled-coil is encoded by a combination of the type-2 exon and a part of the type-3 exon.

B/ Schematic representation of the open reading frame of the NBPF1 gene as predicted from the human genome sequence (NT_004873.16) and present in an isolated cDNA sequence (AY894575) derived from this gene. The isolated cDNA contains multiple copies of exon types 10, 11, 12 and 13, which are present only once in the genomic sequence. The several exon couples that constitute the NBPF/DUF1220 repeat are shown in white boxes at the bottom.

C/ Amino acid alignment of the different NBPF repeats present in the NBPF1 protein. A homologous sequence was isolated from the draft version of the bovine genome and assembled in silico (depicted as AC157123). The bottom sequence is derived from one of the human homologs of rat myomegalin (AB007923; AA 1564-1654). The black line denotes the exon boundaries, whereas the red lines denote the PFAM protein domain of unknown function, DUF1220 (Adapted from Vandepoele et al., 2005, Oxford Journals (2005), reproduced by permission).
be enriched in spliced transcripts (She et al., 2008) and show a higher sequence identity (Marques-Bonet & Eichler, 2009). It has been shown that segmental duplications, together with retrotransposons, account for the large-scale structural variations in primate genomes. Humans and other great apes share interspersed duplications, but a burst of segmental duplications also occurred in the common ancestor of humans and African great apes (Marques-Bonet et al., 2009a).

As segmental duplications appeared quite recently, it is presumed that genes residing in these regions played a role in evolution. The morpheus gene family, for example, is found only in humans and African apes and is located in segmental duplications on chromosome 16 (Johnson et al., 2001). These genes were repeatedly duplicated and positively selected for during primate evolution and, therefore, presumably played a role in speciation events. Amino acid replacements in putative protein-coding exons of this gene family occurred after the separation of human/great-ape lineages from orangutan and after the divergence of human and chimpanzee lineages. This points to adaptive evolution of the morpheus gene family during the emergence of humans and African apes (Johnson et al., 2001). It is becoming clear that lineage-specific gains and losses in gene copy number have emerged as an important aspect of primate genetic variation (Cheng et al., 2005). An array-based comparative genomic hybridisation that surveyed gene duplication and losses across 10 primate species identified over 4,000 genes that show lineage-specific copy-number gains and losses (Dumas et al., 2007). Those genes exhibiting lineage-specific copy number changes are likely involved in the phenotypic differences that distinguish these primate lineages. Interestingly, NBPF/DUF1220 sequences show human lineage-specific copy number increases (Dumas et al., 2007), consistent with previous reports (Fortna et al., 2004; Vandepoele et al., 2005; Popesco et al., 2006). Different experiments indicated that the number of NBPF/DUF1220 repeats was very large in humans, reduced in African great apes, further reduced in orangutan and Old World monkeys, single-copy in non-primate mammals, and absent in non-mammalian species (Popesco et al., 2006). The dramatically elevated copy number in humans indicates the importance of the NBPF/DUF1220 repeat in human evolution. Moreover, comparison of the human chromosome 1 with that of chimpanzee revealed a remarkable human 3-mer higher order repeat (HOR) organisation based on an ~1.6-kbp primary repeat unit fully embedded within the NBPF genes (Figure 4A). This HOR pattern is not found in chimpanzee and shows some peculiarities, namely that the repeat unit is much longer than most primary repeat units identified so far and that the HOR is fully embedded within a gene. Additionally, the total absence of tandem repeats of NBPF HOR copies in chimpanzees while 47 tandem repeat HOR copies are present in human genomes reflects a human accelerated HOR pattern that distinguishes humans from nonhuman primates (Figure 4B) (Paar et al., 2011).

It has been shown that genes with an important contribution to human evolution, like the NBPF gene family, are located in human segmental duplications, which are frequently organised around core duplicons (Jiang et al., 2007). Comparison of chimpanzee and man shows that new lineage-specific segmental duplications map preferentially near shared ancestral duplications, a phenomenon called duplication shadowing (Cheng et al., 2005). Unique genes mapping near these duplication blocks have a ten-fold higher probability of becoming duplicated than other randomly distributed regions. The NBPF/DUF1220 domains are among the few core duplicons that exist in the human genome and appear to be responsible for much of the duplicated sequences in the pericentromeric region of chromosome 1 (Jiang et al., 2007). These large, high-identity duplication blocks are prone to rearrangements that lead to the formation of both harmless and pathogenic copy number variations, as discussed in the following section.

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3.3 The NBPF gene family shows frequent copy number variations that are sometimes associated with pathologies, including neuroblastoma

As previously discussed, primate segmental duplications are larger, more complex and more interspersed than those in other mammalian genomes. This pattern promotes genomic instability and leads to considerable copy number variation (Bailey & Eichler, 2006). A copy number variation (CNV) is a structurally variable region of DNA longer than 1 kbp present in the genomes of humans (Redon et al., 2006), primates and many other species (Graubert et al., 2007; Chen et al., 2009). In these genomes, the DNA region is present in various copy numbers due to gains and losses of genomic DNA. Surprisingly, as much as 12% of the human genome is copy number variable, and despite efficient DNA repair, the rates of de novo CNVs are at least 100 to 10,000 fold greater than rates of point mutations (Lupski, 2007).

Nonallelic homologous recombination between low-copy repeats that flank unique genomic segments changes the organisation of the genome and is thought to be one of the main mechanisms that give rise to CNVs (Inoue & Lupski, 2002). Several studies indicate that the
The NBPF Gene Family

NBPF gene family is polymorphic within the human population (Database of Genomic Variants). Analysis of the copy number differences between the genomes of 159 human individuals showed that the NBPF gene family is one of the most variable gene families in humans (Sudmant et al., 2010). Sudmant et al. (2010) also showed that the NBPF gene duplicates are underrepresented in the human reference genome because no individual examined exhibited a copy number less than or equal to the copy number in the reference genome. Paralog-specific copy number genotyping distinguished two distinct classes of paralogs: one group is mostly copy-invariant in humans, and the other group is extensively copy number variable with some bias toward gain or loss. The NBPF gene family showed a paralog-specific copy number variation, and certain paralogs (NBPF1) were frequently amplified, extremely variable, and stratified by population, whereas other paralogs (NBPF7) were nearly fixed and diploid (Sudmant et al., 2010). Taken together, these data clearly show that the NBPF gene family is polymorphic in the human population.

The functional implications of these structural variations in the human genome can be of great importance: not only are they an important source of genetic diversity between individuals, they can also affect an individual’s susceptibility to disease. Several diseases, such as lupus with glomerulonephritis and Crohn’s disease, have been linked to CNV of FCGR3B and HBD-2, respectively. It has been shown that a low copy number of FCGR3B (Fc fragment of IgG, low affinity IIIb, receptor) predisposes to glomerulonephritis, an immunologically mediated renal disease (Aitman et al., 2006), whereas individuals with less than three copies of the human beta-defensin 2 (HBD-2) gene have a significantly higher risk of developing Crohn’s disease than individuals with four or more copies (Fellermann et al., 2006). In neuroblastoma, an inherited copy number variation was shown to be associated with this childhood cancer. Indeed, Diskin et al. (2009) found that a common deletion polymorphism spanning less than 145 kbp at 1q21.1 is associated with neuroblastoma. Validation by quantitative PCR, fluorescent in situ hybridisation, and analysis of matched tumour specimens showed that this CNV is a heritable genetic variation. Interestingly, the 5'UTR of NBPF17P (NBPF23; see above) is located in this CNV. Quantitative PCR showed that this NBPF paralog is expressed in a variety of fetal and adult tissues, but the strongest expression was observed in fetal brain and fetal sympathetic nervous tissue. Importantly, these experiments showed that the expression level was strictly correlated with the CNV state in neuroblastoma cells (Diskin et al., 2009). Recently, it has become clear that CNVs in the 1q21.1 region are associated not only with neuroblastoma, but also with an increasing number of other pathologies. Intriguingly, these copy number gains or losses either encompassed or flanked members of the NBPF gene family, emphasising the possible involvement of the NBPF gene family in human disease. CNVs at these genomic loci have been found in patients with mental retardation and congenital anomalies (Mefford et al., 2008), autism (Sharp et al., 2006; Szatmari et al., 2007) and schizophrenia (Stefansson et al., 2008). Autism and schizophrenia can be described as diametric conditions because reciprocal variants of the same genomic sequences, namely duplication and deletion, represent risk factors for these respective psychiatric conditions. Additionally, autism and schizophrenia are associated with developmentally enhanced and reduced brain growth, respectively (Crespi et al., 2009). A study of 1q21.1 CNVs and brain size found that deletions of the 1q21 region are associated with microcephaly, which is characterised by an abnormally small head, whereas duplications of this region are associated with macrocephaly, which is characterised by an abnormally large head (Brunetti-Pierri et al., 2008). Notably, the most striking trend in human evolution is the rapid increase in brain size.
over the past 3–4 million years, and the associated increase in complexity and cognitive capacity (Mekel-Bobrov et al., 2007). Because NBPF/DUF1220 is present in remarkably more copies in the human genome than in other primates (Vandepoele et al., 2005; Popesco et al., 2006), it was hypothesised that the NBPF/DUF1220 copy number is correlated with brain size (Dumas & Sikela, 2009). Moreover, these data support the hypothesis that NBPF/DUF1220 domains are involved in the difference in brain size between autistic and schizophrenic populations and between macrocephalic and microcephalic populations. Therefore, NBPF/DUF1220 repeats could have played an important role in human evolution and in the associated increase in brain size. On the other hand, the selective advantage conferred by the increased number of NBPF/DUF1220 domains might have favored retention of the highly unstable 1q21.1 region, resulting in many recurrent rearrangements and leading to a spectrum of disorders of the human brain and to developmental defects (Dumas & Sikela, 2009).

4. **NBPF promoter analysis**

Gene duplication has generally been viewed as a primary mechanism for the origin of evolutionary novelties (Ohno, 1970). In most cases, duplication is not limited to the coding sequence but encompasses the regulatory region as well. Alternatively, new genes can acquire a regulatory region from a nonhomologous gene (Usakin et al., 2005). We found evidence that the regulatory region of the NBPF1 gene was obtained from an unrelated gene, called EVI5 (ecotropic viral integration site 5) (Vandepoele et al., 2009). EVI5 was originally identified through its involvement in a constitutional chromosome translocation in a patient with stage 4S neuroblastoma (Roberts et al., 1998). EVI5 is a centrosomal protein during interphase, but it relocalises to the midbody during late phases of mitosis. Its disruption leads to incomplete cell division and formation of multinucleate cells (Faitar et al., 2005). EV15 also exists in a protein complex with GTP-bound Rab11 and functions as a GTPase-activating protein for this small GTPase (Dabbeekeh et al., 2007). Additionally, EVI5 is critical during interphase for the regulation of cyclin accumulation. It functions by stabilising the anaphase-promoting complex inhibitor, Emi1. RNA interference with EVI5 levels resulted in cell-cycle arrest and abnormal centrosome numbers in vivo, suggesting that EVI5 might not only regulate cyclin accumulation during interphase but also contribute to timing mechanisms to ensure mitotic fidelity (Eldridge et al., 2006).

The human EVI5, located on chromosome 1p22, is transcribed as two isoforms differing in their transcription initiation sites and first exons. Intriguingly, the promoter of NBPF1 shows homology only to the promoter used to control the expression of human isoform 2 of EVI5 (called EVI5_1p22), but not the promoter of isoform 1 (called EVI5_ALT) (Figure 5) (Vandepoele et al., 2009).

The extensive sequence homology in the promoter regions of different NBPF paralogs shows that the recruitment of the promoter from EVI5_1p22 occurred before expansion of the NBPF gene family. Phylogenetic analysis of the NBPF and EVI5_1p22 core promoter sequences showed that promoter duplication occurred after the split between simians and prosimians, but before divergence between Old World and New World monkeys, and that positive selection played a role in the evolution of the NBPF1 promoter in simians (Vandepoele et al., 2009).
Fig. 5. Sequence homology between the promoter regions of different NBPF paralogs and the promoter regions of EVI5. Expression of the human EVI5 gene is controlled by two promoters: EVI5\text{\textsc{alt}} controls the expression of isoform 1 and EVI5\text{\textsc{nbpf}} controls the expression of isoform 2. Arrows indicate the positions of these transcription initiation sites on the reverse strand of chromosome 1, and their first exons are represented by blue boxes on the top line. A region of 20 kbp flanking the transcription initiation site of human and mouse EVI5\text{\textsc{nbpf}} and different NBPF paralogs was aligned by the AVID algorithm in the Vista Genome browser. The promoter of NBPF1 and its paralogs shows homology only with the promoter of human EVI5 that is used to control the expression of isoform 2 (EVI5\text{\textsc{nbpf}}) but not the one for isoform 1 (EVI5\text{\textsc{alt}}). The homology between the human and mouse EVI5 sequences is confined mainly to the two regions corresponding to the putative promoter regions. No official gene symbol is available for NBPF\textsuperscript{**} (Adapted from Vandepoele et al., 2009, Oxford Journals (2009), reproduced by permission).

The expression patterns of NBPF and EVI5 in a panel of human cell lines are very similar, and the expression of both genes was decreased in neuroblastoma cell lines in which there is 1p36 loss of heterozygosity. Nevertheless, the mechanism of this decreased expression level was due to use of a different transcriptional and posttranscriptional regulation. Whereas EVI5 was epigenetically silenced, treatment of the neuroblastoma cell lines with puromycin, an inhibitor of nonsense-mediated decay, indicated that mutation of the NBPF transcripts was probably responsible for decreasing the expression levels. These data together show that despite the almost identical promoter sequences and very similar expression patterns, regulation of NBPF and EVI5 genes in neuroblastoma cell lines can occur by different mechanisms (Vandepoele et al., 2009).
5. NBPF-interacting proteins

Many genes perform their functions by interaction of their encoded proteins with other proteins. The search for NBPF-interacting proteins revealed two hits so far: chibby and clusterin, which together can form a tri-molecular complex with NBPF1 (Vandepoele et al., 2010). Interestingly, both NBPF1 and clusterin are candidate tumour suppressors for neuroblastoma (Vandepoele et al., 2008; Chayka et al., 2009). This suggests that the interactions between these three proteins are functionally important.

5.1 Chibby

Chibby (Cby) is a 15-kDa protein that has been strongly conserved throughout evolution from fly to human. It was originally identified as an inhibitor of the canonical Wnt-β-catenin pathway (Takemaru et al., 2003). Cby interacts with the C-terminal activation domain of β-catenin and blocks its transcriptional activation potential by two mechanisms. First, it competes with T cell factor/lymphoid-enhancer factor (TCF/LEF) transcription factors for binding with β-catenin (Takemaru et al., 2003). Second, in cooperation with 14-3-3 adaptor proteins, it facilitates nuclear export of β-catenin (Li et al., 2008). Inhibition of the Wnt-β-catenin pathway is necessary for cardiomyocyte differentiation, proliferation and repair, and Cby has been implicated in facilitating cardiomyocyte differentiation (Singh et al., 2007). Additionally, several molecules of the Wnt-β-catenin pathway play important roles in the inhibition of adipogenesis, and different findings indicate that Cby is required for adipocyte differentiation (Li et al., 2007). Cby also plays a role in other processes. Reports on the binding of Cby to polycystin-2 (Hidaka et al., 2004) and on the Cby knockout mouse (Voronina et al., 2009; Love et al., 2010) implicate Cby in the development of motile airway cilia. Germline inactivation of Cby resulted in complete absence of mucociliary transport due to scarcity of motile cilia in the nasal epithelium (Voronina et al., 2009). In a follow-up study, characterisation of the lung morphology of Cby knockout mice showed that Cby-/- lungs were normal at birth, but alveolar airspace became enlarged, proliferation became reduced, and lung epithelial cells differentiated abnormally, which together affected pulmonary function (Love et al., 2010). Both the lungs and nasal epithelia of Cby knockout mice showed increased expression of two direct β-catenin target genes, namely Axin2 and CyclinD1, but it is not clear whether the ciliary phenotypes are related to the modest elevation of these Wnt related genes, since no change in the localisation of certain components of the Wnt-β-catenin pathway was observed. Presence of endogenous Cby at the base of cilia and the phenotypes related to the loss of Cby show that Cby is important in ciliogenesis (Voronina et al., 2009; Love et al., 2010).

Neuroblastoma is a disease of the sympathoadrenal lineage of the neural crest, and these tumours can develop anywhere in the sympathetic nervous system. At least three key pathways regulate the formation, migration and differentiation of the neural crest cells: the bone morphogenetic protein (BMP) pathway (Barembaum & Bronner-Fraser, 2005), the fibroblast growth factor pathway (Sauka-Spengler & Bronner-Fraser, 2008) and the Wnt signalling pathway (Crane & Trainor, 2006). Wnt signalling plays an important role in neural crest cells. For example, mouse embryos in which Wnt1 and Wnt3 were knocked out displayed severe loss of neural crest cells (Varga & Wrana, 2005). In addition, conditioned medium of cells overexpressing wingless (Drosophila homolog of Wnt1) could induce avian neural crest cells (Garcia-Castro et al., 2002). Aberrant regulation of the embryonic developmental Wnt pathway has been implicated in many solid childhood tumours (reviewed in Koesters & Doeberitz, 2003), but a clear role for Wnt signalling in...
neuroblastoma has remained elusive. Nevertheless, there is mounting evidence that the Wnt pathway is involved in neuroblastoma (Table 1).

The MSX1 homeobox transcription factor functions as an intermediate between the BMP and Wnt signalling pathways to ensure proper differentiation of the neural crest cells. Elevated expression of BMP in non-neuronal ectoderm switches on the neural crest differentiation program and induces the expression of MSX1, which leads to induction of the expression of BMP and Wnt in neural crest cells (Ramos & Robert, 2005). Msx genes might regulate Wnt1 expression at the dorsal midline of the neural tube (Bach et al., 2003).

Additionally, MSX1 overexpression in the MYCN-amplified neuroblastoma cell line SJNB8 induced the expression of four Wnt inhibitor genes: Dickkopf 1 (DKK1), Dickkopf 2 (DKK2) and Dickkopf 3 (DKK3) and secreted frizzled-related protein 1 (SFRP-1) (Revet et al., 2010). Analysis of the expression profiles of MSX1, DKK1, -2, -3 and SFRP-1 in a series of neuroblastic tumours showed a significant and positive correlation between MSX1 and DKK2 expression and between MSX1 and DKK3 expression. Additionally, stronger DKK2 and DKK3 expression correlated with a significantly better prognosis. Expression profiling of neuroblastic tumours and cell lines for the different Wnt family genes showed that both the canonical Wnt3a and the non-canonical Wnt5 were strongly expressed and that they could activate upstream Wnt signalling in neuroblastoma cells by phosphorylating the dishevelled co-receptor DVL3 (Revet et al., 2010). The importance of DKK3 in neuroblastoma was already shown in previous reports, in which DKK3 was implicated as a marker for neuroblastic tumour maturation and was shown to be down-regulated by MYCN (Bell et al., 2007; Koppen et al., 2008).

Additionally, β-catenin has been shown to be strongly expressed and aberrantly localised in the nucleus in high-risk neuroblastoma cells without MYCN amplification (Liu et al., 2008). Expression profiling of primary neuroblastoma tumours demonstrated increased expression of WNT ligands (WNT1, WNT6, WNT7A, WNT10B), DVL1 and TCF7 in high-risk neuroblastoma tumours without MYCN amplification. Also several β-catenin target genes, such as CD44, MDR1 and FZD1, were increased in chemoresistant NB cell lines and in relapsed patients.

Table 1. Wnt signalling is important in neuroblastoma tumourigenesis and chemoresistance. Schematic representation of different Wnt signalling components implicated in neuroblastoma. NB: neuroblastoma; MNA: MYCN amplification. See text for references.
e.g. MYC and CD44, were found to be coordinately upregulated in high-risk neuroblastomas without MYCN amplification in comparison to high-risk MYCN-amplified or intermediate-risk neuroblastomas (Liu et al., 2008). Another study implicated the Wnt-β-catenin pathway in neuroblastoma chemoresistance (Flahaut et al., 2009). Gene expression profiling of two doxorubicin-resistant neuroblastoma cell lines identified the frizzled1 Wnt receptor (FZD1) gene as the most strongly overexpressed transcript in both cell lines in comparison with their sensitive parental cell lines. FZD1 silencing resulted in significant restoration of drug sensitivity and induced a parallel strong decrease in the expression of MDR1 (Flahaut et al., 2009), another β-catenin target gene frequently associated with the resistance of neuroblastoma tumours to chemotherapeutic drugs (Haber et al., 1997). Moreover, FZD1 and/or MDR1 expression was significantly enhanced in the group of relapsed patients after chemotherapy, whereas no significant increase in expression was measured in the group of non-relapsed patients (Flahaut et al., 2009).

These data indicate that Wnt signalling is important in neuroblastoma tumourigenesis and chemoresistance (overview in Table 1). Therefore, we hypothesise that important players in the Wnt-β-catenin pathway might play important roles in neuroblastoma. But nothing is known yet about the role of Cby in neuroblastoma tumourigenesis or chemoresistance, and further investigation is needed.

5.2 Clusterin
Clusterin (CLU), also known as apolipoprotein J, is a heterodimeric sulfated glycoprotein that is expressed in most human tissues and body fluids and is associated with many biological activities, including regulation of apoptosis and cancer (Trougakos & Gonos, 2002). Research on CLU function and its relation to tumourigenesis has been intensive, but many contradictory data have been reported. These contradictions can be explained mainly by the existence of three protein isoforms with different sub-cellular localisations and biological functions (Rizzi & Bettuzzi, 2010). The most extensively studied form of CLU is the secreted form (sCLU), which is a glycosylated heterodimer present in almost all physiological fluids. sCLU is exported from the cell and released in secretions, in which it acts as an extracellular chaperone (Humphreys et al., 1999). Cytoplasmic clusterin has been shown to inhibit apoptosis by interfering with Bax activation in the mitochondria (Zhang et al., 2005). Clusterin binds and stabilises the Ku70-Bax protein complex and serves as a cytosolic retention factor for Bax. Depletion of clusterin disrupts the Ku70-Bax complex and triggers Bax activation and its relocalisation to the mitochondria (Trougakos et al., 2009). Cytoplasmic clusterin also promotes tumour cell survival by cooperating with c-Myc during transformation (Zhang et al., 2005). Additionally, a large amount of CLU confers resistance to cytotoxic agents on different cancer cell types (Lourda et al., 2006). However, a nuclear form of clusterin originating from an alternative translation start site has been shown to be proapoptotic in prostate cancer cells (Moretti et al., 2007), breast cancer cells (Leskov et al., 2003) and neuroblastoma (Yang et al., 2000). The main question is whether clusterin is a positive or negative modulator of mammalian tumourigenesis. It is becoming clear that CLU can act either as a tumour suppressor (Caporali et al., 2004; Chayka et al., 2009) or as a tumour promoter (Lourda et al., 2006), but the time-course of the disease and the selection pressures imposed on the cancer by chemotherapy or other treatments have to be taken into account.
Clusterin knockout mice are more prone to autoimmune myocarditis (McLaughlin et al., 2000) and to transformation of the prostate epithelium (Bettuzzi et al., 2009). In homozygous knockout mice, the normal portion of prostate tissue is characterised by stronger expression of the proliferation marker Ki67 and shows activated NF-κB signalling, but this was not observed in wild type mice (Bettuzzi et al., 2009). Additionally, in TRAMP mice, a transgenic animal model of prostate cancer (Greenberg et al., 1995), the CLU gene is dramatically downregulated during onset and progression of prostate cancer (Caporali et al., 2004). Crossing of these TRAMP mice with the clusterin knockout mice led to development of a more advanced invasive disease in the progeny, confirming the role of CLU as a negative tumour modulator in prostate cancer (Bettuzzi et al., 2009).

On the contrary, overexpression of clusterin in LNCaP prostate cancer cells conferred resistance to both androgen ablation in vivo and cytotoxic chemotherapy (Miyake et al., 2000c). Additionally, the administration of clusterin antisense oligonucleotides in prostate cancer xenograft models delayed progression to androgen independence and enhanced chemosensitivity (Miyake et al., 2000b). A human clusterin antisense oligonucleotide (OGX-011) synergistically enhanced the cytotoxic effects of paclitaxel in human xenografts of prostate cancer (Greenberg et al., 1995), and this antisense oligonucleotide is currently undergoing clinical trials for tumour therapy. The phase II trial demonstrated that OGX-011 is well tolerated and that it inhibits clusterin expression in prostate cancer (Chi et al., 2005). The phase III trial assessed the effects of combined therapy with androgen ablation and OGX-011 given prior to radical prostatectomy in patients with metastatic castration-resistant prostate cancer. This study showed that the combined therapy was associated with improved survival (Chi et al., 2010).

The biological role of clusterin is controversial in both prostate cancer and neuroblastoma. Indeed, also in neuroblastoma it has been shown that clusterin can function both as a tumour suppressor gene and as an oncogene. Overexpression of clusterin impaired the invasion of neuroblastoma cell lines by inhibiting the NF-κB activity by stabilising IκBs, the inhibitors of NF-κB signalling (Santilli et al., 2003). Additionally, Oncomine expression analysis (Rhodes et al., 2007) showed a lower expression level of the CLU gene in primary human neuroblastomas with MYCN amplification, and further investigation demonstrated that MYCN acts as a negative regulator of clusterin expression by inducing transcription of the 17-5p-92 microRNA cluster (Chayka et al., 2009). This polycistronic cluster encodes six microRNAs (miR-17-5p, -18a, -19a, -19b, -20a and -92) and is homologous to the miRNA 106a-92 cluster (Mestdagh et al., 2010b). Most miRNAs encoded by this cluster are expressed at higher levels in neuroblastoma cell lines overexpressing MYCN or carrying MYCN amplification. MYCN transcriptionally induces the expression of the miRNA 17-5p-92 cluster by directly binding to its promoter. The therapeutic potential of this miRNA cluster was demonstrated by injecting a miR-17-5p-specific antagonim into nude mice that had been injected with a neuroblastoma cell line with MYCN amplification. Tumour growth in vivo was reduced substantially, and this reduction was associated with down-modulation of miR-17-5p activity and with a reciprocal increase of both p21 and BIM protein levels. Nevertheless, the expression pattern of miR-17-5p in primary tumours is complex, because high levels of miR-17-5p expression were detected in both MYCN-amplified and non MYCN-amplified tumours (Fontana et al., 2008). Additionally, it has been demonstrated that miR-17-5p-92 dampens TGF-β signalling by acting both upstream and downstream of pSMAD2/SMAD4 (Mestdagh et al., 2010a). This finding further underscores its ability to regulate multiple components of the same pathway.
When MYCN-transgenic mice were crossed with clusterin knockout mice, the progeny showed reduced tumour-free survival, active NF-κB signalling, and epithelial to mesenchymal transition (Chayka et al., 2009); this resembles the phenotype of TRAMP mice in which CLU was homozygously deleted (Bettuzzi et al., 2009). These data indicate that clusterin functions as a TSG for neuroblastoma, even though several reports indicate that it can promote neuroblastoma. Treatment with histone deacetylase inhibitors (HDACIs) resulted in upregulation of clusterin in different cancer cell lines, including neuroblastoma (Liu et al., 2009; Subramanian et al., 2011). Suppression of clusterin in combination with high-dose HDACIs synergistically enhanced HDACI-induced cell death through cytochrome-c-mediated apoptosis in HDACI-resistant cancer cells. Additionally, combining OGX-011 with low-dose HDACIs enhanced HDACI-induced growth arrest in both HDACI-sensitive and -resistant cancer cell lines. Moreover, in mice xenografted with neuroblastoma cells, combining OGX-011 and valproate (the only HDACI proven to be safe in young children) synergistically inhibited tumour growth in vivo, whereas neither OGX-011 nor valproate alone had an effect on tumour progression (Liu et al., 2009).

![Fig. 6. Proposed model for CLU in tumourigenesis.](image_url)

In normal cells and under physiological conditions the basal expression level of CLU in benign cells is low and confined to the secreted form of CLU (sCLU) (1). Several apoptotic stimuli (2) lead to the production of nuclear CLU (nCLU) (3), which in turn induces extensive cell death (4). In the early phases of tumourigenesis, genetic lesions lead to acquisition of survival advantages and consequent clonal selection (5). A key step in the transformation process is prevention of CLU from entering the nucleus. Cells with high levels of cytoplasmic CLU are then selected for, which results in acquisition of a death-resistant phenotype (6). This phenotype is associated with impaired mitosis (7), which leads eventually to genomic instability (8) (Modified after Rizzi & Bettuzzi, 2010, Society for Endocrinology (2010), reproduced by permission).
Based on these findings, which were observed in both prostate cancer and neuroblastoma, a role for clusterin in tumourigenesis has been proposed (Figure 6). This model proposes that clusterin acts as a tumour suppressor in the early stages of cancer but can become a tumour promoter in the more malignant and advanced stages, namely when tumours become resistant to therapy (Rizzi & Bettuzzi, 2010). Under physiological conditions, the basal expression level of CLU in benign cells is low and confined to the secreted form of CLU (sCLU). Several apoptotic stimuli lead to the production of nuclear CLU (nCLU), which in turn induces extensive cell death. At the onset of cancer, early genetic lesions disrupt or inactivate apoptotic pathways or lead to the activation of other survival pathways. Under these conditions of clonal selection, a key step in the transformation process would be the prevention of CLU from entering the nucleus in order to prevent induction of apoptosis. At this point, cancer cells are characterised by strong expression of cytosplasmic CLU (cCLU) and are therefore resistant to death by cytotoxic therapeutic agents. This resistant phenotype can promote cell transformation that leads to late cancer stages associated with impaired mitosis, which causes further genomic instability (Rizzi & Bettuzzi, 2010).

5.3 NBPF1, Chibby and clusterin can form a tri-molecular complex
A yeast two-hybrid screening with an N-terminal fragment of the NBPF11 protein identified Cby as a binding partner of NBPF11 (Vandepoele et al., 2010). This interaction is not exclusive to NBPF11 but is common to other NBPF members. For instance, Cby can also interact with NBPF1. The interaction was confirmed by other techniques, such as co-immunoprecipitation and mitochondrial recruitment (Vandepoele et al., 2010). It was shown that the coiled-coil region in the N-terminal domain of NBPF interacts with the coiled-coil region in the C-terminal domain of Cby. This C-terminal portion of Cby harbours not only the binding site for NBPF, but also the binding site for most other known interaction partners of Cby, such as β-catenin (Takemaru et al., 2003), polycystin-2 (Hidaka et al., 2004) and TC1 (Jung et al., 2006).

Functional analysis of the interaction showed that NBPF did not influence Cby-mediated Wnt signalling, nor did it compete with β-catenin binding to Cby. This indicates the presence of two different pools of Cby, one binding to β-catenin and the other binding to NBPF, or alternatively, a dimeric Cby complex that can bind simultaneously to β-catenin and NBPF. Besides its repressor function in the Wnt pathway, Cby can also play an important role in ciliogenesis, as shown in the Cby knockout mouse (see section 5.1). The role of NBPF in the formation of cilia remains uncertain, as NBPF1 overexpression did not interfere with the formation of primary cilia (Vandepoele et al., 2010). An additional yeast two-hybrid screen identified clusterin as a new binding partner for Cby and showed that the C-terminus of CLU, which contains a coiled-coil domain, interacts with both the N-terminal and C-terminal domains of Cby. Additionally, co-immunoprecipitation experiments in HEK293T cells demonstrated that NBPF1, Cby and clusterin can form a tri-molecular complex (Figure 7), because all three proteins were co-purified in every setting. Since intracellular CLU can be found both in the nucleus and in the cytoplasm (see section 5.2), the effect of Cby or NBPF or their combination on the subcellular localisation of CLU was further investigated, but no difference was observed (Vandepoele et al., 2010). The identification of two proteins that interact with NBPF is a clue to the functions of NBPF, but further investigation is needed. Anyway, the identification within the complex of two proteins (NBPF1 and CLU) as candidate tumour suppressor genes linked to neuroblastoma (Vandepoele et al., 2008; Chayka et al., 2009) is intriguing and warrants further scrutiny.

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An overview of the mutual interactions between NBPF1, Cby and CLU. The coiled-coil region in the N-terminus of NBPF1 interacts with the coiled-coil region in the cytoplasmic domain of Cby. The C-terminus of CLU interacts with the full-length Cby protein, as interactions with both the N-terminus and C-terminus are observed. It is uncertain whether CLU and NBPF1 interact directly (boxed question mark) (Modified after Vandepoele et al., 2010, Elsevier (2010), reproduced by permission).

6. Conclusion

In this chapter we summarise the growing evidence for the recent emergence of the NBPF gene family and for its role in human evolution. The NBPF/DUF1220 domains in particular seem to have been important in this context. This evidence is based on the large increase in copy number of the NBPF/DUF1220 domain in humans. On the other hand, NBPF genes seem to be involved in cancer and in brain and developmental disorders due to their location in unstable high-identity duplication blocks, which leads to recurrent chromosomal rearrangements. One case of particular interest is neuroblastoma. Evidence for the involvement of NBPF genes in this type of cancer comes not only from disruption of NBPF1 in a neuroblastoma patient, but also from gene expression studies and the association with neuroblastoma of a copy number variation of an NBPF paralog. Additionally, the regulatory elements and the identification of NBPF-interacting proteins indicate that the NBPF gene family plays a role in neuroblastoma. Involvement of NBPF genes in neuroblastoma and possibly as well in other cancers points to the value of studying this gene family further. Every step in the functional understanding of these genes could bring us closer to unravelling the molecular mechanisms underlying neuroblastoma development and could help us to develop more effective therapy. Such studies could also shed light on the impact these novel genes had on recent primate evolution.

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8. References

The NBPF Gene Family


The NBPF Gene Family


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Neuroblastoma, once called "enigmatic", due to "unpredictable" clinical behaviors, is composed of biologically diverse tumors. Molecular/genomic properties unique to the individual tumors closely link to the clinical outcomes of patients. Establishing risk stratification models after analyzing biologic characteristics of each case has made a great success in patient management. However, the trend of improving survival rates in neuroblastoma over the last 30 years has started to level off, and currently available treatment modalities have almost reached to their maximized intensity. Furthermore, aggressive treatment causes significant long-term morbidities to the survivors. We really need to make the next step to the level of personalized medicine with more precise understanding of neuroblastoma biology. This book includes useful data and insights from the world's experts in this field. I believe this book can make an excellent contribution to all the investigators working hard and fighting for the children stricken by this disease.

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