Chapter 12
The Adhesion Molecule Anosmin-1 in Neurology: Kallmann Syndrome and Beyond

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Abstract Anosmin-1 is the glycoprotein encoded by the *KAL1* gene and part of the extracellular matrix, which was first identified as defective in human Kallmann syndrome (KS, characterised by hypogonadotropic hypogonadism and anosmia); biochemically it is a cell adhesion protein. The meticulous biochemical dissection of the anosmin-1 domains has identified which domains are necessary for the protein to bind its different partners to display its biological effects. Research in the last decade has unravelled different roles of anosmin-1 during CNS development (axon pathfinding, axonal collateralisation, cell motility and migration), some of them intimately related with the cited KS but not only with this. More recently, anosmin-1 has been identified in other pathological scenarios both within (multiple sclerosis) and outside (cancer, atopic dermatitis) the CNS.

12.1 Introduction

Anosmin-1 is the protein encoded by the *KAL1* gene. This is a glycoprotein of the extracellular matrix which was first identified as defective in human Kallmann syndrome (KS) characterised by hypogonadotropic hypogonadism and anosmia.

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Its biochemical characteristics allow for its classification as a cell adhesion protein. Beyond all these classical facts about anosmin-1, research in the last decade has involved the protein in different aspects of central nervous system (CNS) development, some of them related with the cited KS but not only with this and, more recently, anosmin-1 has been identified in other pathological scenarios, outside the CNS, too. In parallel, the meticulous biochemical dissection of the anosmin-1 domains has substantially unravelled which are necessary for the protein to bind receptors and other partners and which are indispensable for anosmin-1 to display its biological effects.

12.2 The *KAL1* Gene and the Protein Anosmin-1: Structure, Expression and Regulation

12.2.1 The KAL1 *Gene*

The gene responsible for the X-linked form of KS, *KAL1*, was first identified in 1991 (Franco et al. 1991; Legouis et al. 1991). The *KAL1* gene comprises 14 exons, is located on the X chromosome (Xp22.3), and has an inactive homologous gene on the Y chromosome (del Castillo et al. 1992), which together with the fact that this gene partially escapes X-chromosome inactivation (Franco et al. 1991; Shapiro et al. 1979) would explain the higher prevalence of the disease in males (Dode et al. 2003; Laitinen et al. 2011). Orthologs have been identified in a variety of invertebrate and vertebrate species, from the nematode worm *C. elegans* to rodents and primates, but despite the high degree of sequence identity among species shown by the protein anosmin-1 coded by the gene *KAL1*; no ortholog has been identified in mouse or rat. The *KAL1* locus is adjacent to the pseudoautosomal region 1 (PAR1) from where a 9 Mb block has been removed or translocated from a common ancestor of mouse and rat (Ross et al. 2005) and while the addition of genes to the PAR1 region from autosomes seems to have occurred in eutherians, macropodid marsupials and monotremes; the loss of PAR1 genes from the mouse X-chromosome is evident (Mangs and Morris 2007). Additionally, this genomic region is not stably propagated in bacteria (Perry et al. 2001) and may, in fact, be highly variable (Church et al. 2009). A high proportion of the genes located in the human PAR1 and the proximal Xp22.3 region has not been identified in the mouse genome or is located on autosomal chromosomes (Gläser et al. 1999). The development of the olfactory system is a well-conserved process throughout evolution, from amphibians to primates, requiring a similar molecular mechanism in all of them and, instead of losing the *KAL1* gene during evolution, it has been suggested that the *KAL1* ortholog in mouse is extremely divergent from the human one, or a compensating mechanism has been originated (Lutz et al. 1993).

12.2.2 *The Protein Anosmin-1*

The protein encoded by the *KAL1* gene is anosmin-1, which is secreted to the extracellular matrix (ECM). Anosmin-1 is a 680 amino acid glycoprotein that presents a modular disposition of different domains. It contains a cysteine rich region (CR domain), a whey acidic protein-like (WAP) domain similar to that found in serin protease inhibitors, four fibronectin type III domains (FnIII) present in several other ECM and cell-adhesion molecules and a C-terminal region with a high content of basic residues histidines and prolines (Legouis et al. 1993). There are five potential heparan sulphate binding motifs along the protein sequence that seem to be important for the proper localisation of the protein within the ECM (Robertson et al. 2001) and 6 putative *N*-glycosylation sites (Rugarli et al. 1996) whose functionality and role in anosmin-1 function remains to be elucidated. The proposed extended domain arrangement and flexible interdomain connections would make anosmin-1 present large basic charged surfaces within the FnIII repeats that could be important in the binding to heparan sulphates and to other interacting proteins (Robertson et al. 2001).

12.2.3 *Expression of* KAL1 *and Anosmin-1. Regulation of the Expression of the* KAL1 *Gene*

In humans, the pattern of expression of the *KAL1* gene and anosmin-1 has been established by in situ hybridisation and immunohistochemistry. *KAL1* transcripts are detected as early as 5 weeks of development, and the expression of the protein has been described as restricted to basement membranes and interstitial matrices of discrete embryonic areas, as should be expected for an ECM protein. Structures that express *KAL1* include the developing olfactory bulb (OB), retina, cerebellum, spinal cord, inner ear and kidney, correlating with the distribution of clinical significant abnormalities in KS patients (Duke et al. 1995; Hardelin et al. 1999; Lutz et al. 1994). Although in $GnRH^+$-neurons *KAL1* transcripts and anosmin-1 have not been detected, both are present in the olfactory nerve fibres and migratory pathways followed by $GnRH^+$-neurons in their way to the hypothalamus, in close association with these cells (Hardelin et al. 1999).

KAL1 expression within the developing olfactory system has been observed in different species. In humans, expression can be seen in the outer olfactory nerve layer of the OB, in olfactory and granule cells as well as in glial cells (Duke et al. 1995; Lutz et al. 1994). In other species such as rat, chicken or zebrafish, expression has been described in the mitral cell layer of the OB, the lateral olfactory tract (LOT) and the olfactory cortex (Ardouin et al. 2000; Ayari and Soussi-Yanicostas 2007; Ayari et al. 2012; Clemente et al. 2008; Dellovade et al. 2003; Lutz et al. 1993; Soussi-Yanicostas et al. 2002; Yanicostas et al. 2009).

Expression has also been described in the cerebellum in human foetus (Lutz et al. 1994) and in several species such as chick, the Asian musk shrew, rat and zebrafish, associated to Purkinje cells, neurons in deep nuclei, the internal granular layer and astrocytes (Ardouin et al. 2000; Ayari et al. 2012; Clemente et al. 2008; Dellovade et al. 2003; Gianola et al. 2009; Legouis et al. 1993; Rugarli et al. 1993; Soussi-Yanicostas et al. 1996, 2002). More recently *KAL1* expression has been shown in muscle, cultured human skeletal muscle cells, basal layer of the skin, dermal cells such as vascular endothelial cells, fibroblasts and cultured human keratonicytes (Raju and Dalakas 2005; Tengara et al. 2010). Anosmin-1 expression has been shown in the germ cells of the testis during spermatogenesis and in the granulosa cells and oocytes in mature ovaries in the marsupial tammar wallaby, which suggests that in addition to the regulatory role in GnRH migration and therefore in the onset of hypogonadotropic hypogonadism, *KAL1* could also work locally in the gonads regulating spermatogenesis and folliculogenesis (Hu et al. 2011).

Apart from the fact that *KAL1* partially escapes X-chromosome inactivation (Franco et al. 1991; Shapiro et al. 1979), little is known regarding the regulation and control of the expression of this gene. In recent years regulation of *KAL1* expression in different tumour tissues and cell lines and inflammatory disorders has been observed, suggesting a possible role of anosmin-1 in the pathogenesis of some of them. Different factors such hypoxia, the methylation of CpG islands in the promoter of the gene and molecules involved in inflammatory processes could be involved in the regulation of the expression of the *KAL1* gene (Arikawa et al. 2011; Jian et al. 2009; Kawamata et al. 2003; Mihara et al. 2006; Raju and Dalakas 2005; Tengara et al. 2010).

12.2.4 Mechanisms of Action of Anosmin-1

Despite recent significant advances, the mode of action of anosmin-1 is not completely comprehended and interactions with different proteins of the ECM, as well as with membrane receptors, have been suggested as major events in the regulation of the activity of this protein. The best known mechanism of action of anosmin-1 is the interaction with the fibroblast growth factor receptor 1 (FGFR1) and the modulation of the activation of this receptor, linking two of the genes responsible for KS (Ayari and Soussi-Yanicostas 2007; Bribián et al. 2006; Dode et al. 2003; García-González et al. 2010; González-Martínez et al. 2004; Hu et al. 2009; Murcia-Belmonte et al. 2010). Interaction between anosmin-1 and FGFR1 has been reported by co-immunoprecipitation (CoIP) (Ayari and Soussi-Yanicostas 2007; Bribián et al. 2006; González-Martínez et al. 2004), and it has been determined that the WAP and FnIII.1 domains together and the FnIII.3 domain by itself interact with FGFR1 (Hu et al. 2009; Murcia-Belmonte et al. 2010). Not surprisingly, some of the missense mutations in the *KAL1* gene found

in KS patients lie within the FnIII domains involved in the interaction with FGFR1 (N267K, E514K, F517L), impeding or greatly reducing the interaction with the receptor and rendering non-functional proteins (Cariboni et al. 2004; Hu et al. 2009; Murcia-Belmonte et al. 2010). Mutations within the WAP domain have also been reported in KS patients, mainly missense mutations replacing some of the cysteine residues and presumably disrupting the formation of disulphide bonds and the correct folding or structure of the domain. An intact WAP domain seems to be required for anosmin-1 biological activity (Bülow et al. 2002; González-Martínez et al. 2004; Hu et al. 2004), but in some scenarios the mutations in the WAP domain do not have a negative effect on the activity of the protein (Bülow et al. 2002; Hu et al. 2004).

Approximately two-thirds of the mutations described in the *KAL1* gene are deletions or frameshift or nonsense mutations that could affect the overall length or composition of the protein, sufficient to account for the disease (Hu and Bouloux 2011), what conflicts with the fact that a truncated N-terminal protein comprising the CR-WAP-FnIII.1 domains, behaves as the full-length protein in some scenarios regarding some of the biological functions of anosmin-1 involving FGFR1 (Bülow et al. 2002; González-Martínez et al. 2004; Hu et al. 2004; Murcia-Belmonte et al. 2010). It has been suggested that the function of the different anosmin-1 domains could be determined or conditioned by the extracellular environment and that the protein could be involved in different phenotypic effects, depending on the cell type or the interaction with different receptors or binding proteins (Andrenacci et al. 2006; Bülow et al. 2002). Therefore, it could be speculated that the biological response exerted by the mutated forms of anosmin-1, could be different from that elicited by the full-length protein or the truncated N-terminal protein lacking the last three FnIII domains, since these proteins could present a different binding capacity to FGFR1 and to other receptors or molecules of the ECM.

The interaction of anosmin-1 with heparan sulphates (HS) present in the ECM seems to be important for the localisation and binding of anosmin-1 to the ECM (Hu et al. 2004; Rugarli et al. 1996; Soussi-Yanicostas et al. 1996), and interaction between anosmin-1 and syndecan-1 and glypican-1 regulates the migration of neuroblasts in the *C. elegans* embryo (Hudson et al. 2006). In fact, it has been shown that the action of anosmin-1 in *C. elegans* is dependent on the presence of HS that contain iduronic-acid and 6O-sulphate groups, but not on HS containing 2O-sulphate groups (Bülow and Hobert 2004). Both FGFR1 and anosmin-1 require heparan sulphate proteoglycans (HSPGs) for their biological functions, since HS are essentials for FGF-FGFR complex formation and receptor activation (Guimond and Turnbull 1999; Hu et al. 2004; Rugarli et al. 1996; Soussi-Yanicostas et al. 1996). It has been hypothesised that due to its diffusible nature, anosmin-1 would exert opposing effects depending on the binding dynamics to FGF2–FGFR1–HS complexes. HS-bound anosmin-1 will associate with pre-formed FGF2–FGFR1 pairs and facilitate FGF2–FGFR1–HS signalling enabling FGFR1-mediated cell migration, the role of anosmin-1 being the presentation of the appropriate HS to the complex.

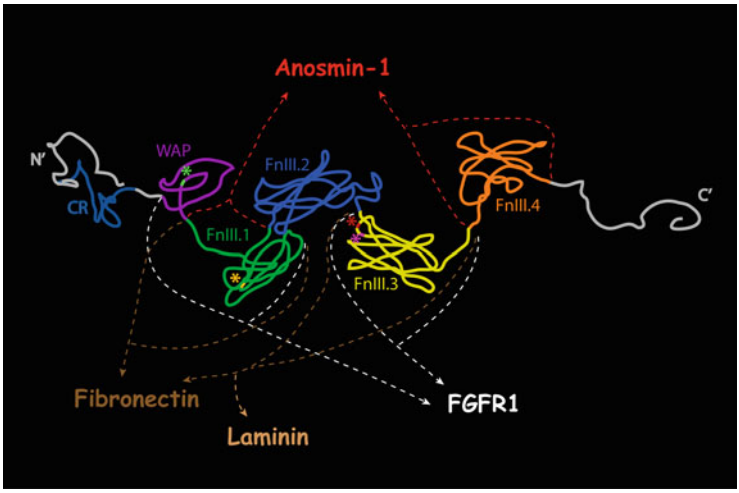

Fig. 12.1 Anosmin-1 interaction map. The WAP-FnIII.1 and FnIII.3 domains allow anosmin-1 to interact with specific sites within FGFR1 and mutations within these domains (N267K in FnIII.1, *orange asterisk*; E514K and F517L in FnIII.3, *red* and *pink asterisk*, respectively) impede this union. The N-terminal truncated protein comprising the CR-WAP-FnIII.1 domains, A1Nt, is still able to bind to the receptor through the WAP and FnIII.1 domains and is biologically functional. On the contrary, in the full-length protein both E514K and F517L substitutions impede the FnIII.3 interaction with FGFR1 and produce a non-functional protein. The N-terminal region would not be able to exert its function correctly, maybe due to the induction by these mutations of conformational changes or to an unstable coupling to the receptor. Mutations in the WAP domain (C172R, *green asterisk*) give rise to functional or non-functional proteins depending on the cellular environment. The FnIII.1 and FnIII.3 domains interact with fibronectin, but only FnIII.3 does interact with laminin. Unlike in the case of FGFR1, the E514K and F517L substitutions do not hinder these interactions and only the F517L mutation reduces the binding to fibronectin. Homophilic anosmin-1/anosmin-1 interactions occur via the FnIII.1 and FnIII.4 domains

In this scenario, the nature of the cell surface HS could be important in determining the anosmin-1-mediated responses. On the other hand, when anosmin-1 levels are high, HS-unbound anosmin-1 could diffuse freely and bind directly to FGFR1 on the cell surface, hampering the formation of the FGF2–FGFR1–HS complex (Hu et al. 2009).

Anosmin-1 has been also shown to interact with other components of the ECM such as uPA (Hu et al. 2004), fibronectin, laminin and anosmin-1 itself (Bribián et al. 2008; Murcia-Belmonte et al. 2010) (Fig. 12.1). Although some of the domains involved in the interaction with FGFR1 participate in the binding to these proteins, the mutations found in FnIII.3 that impede anosmin-1 binding to FGFR1 have little or no effect in the binding to fibronectin and laminin. This, together with the fact that the FnIII.4 domain also participates in the binding to anosmin-1, suggests a mechanism of action independent of FGFR1 (Bribián et al. 2008; Murcia-Belmonte et al. 2010).

12.3 Anosmin-1 Biological Effects

12.3.1 Anosmin-1 in Cell Migration

As a classical ECM protein, anosmin-1 is involved in substrate adhesion and cell migration. This is the case of GnRH-1 neurons, over which anosmin-1 plays a chemoattractive role. Assays performed in GN11 cells (immortalised migrating GnRH-1 human neurons) demonstrated that anosmin-1 with mutations found in KS patients is unable to induce the migration of these cells (Cariboni et al. 2004). This protein is also a chemoattractant cue for neuroblasts migrating out of the subventricular zone (SVZ) on their way to the OB during perinatal development and, together with FGF2, which exerts a motogenic effect; both regulate the migration of these neuronal precursors (García-González et al. 2010). Interestingly, this chemoattraction is mainly mediated through FGFR1 (García-González et al. 2010; Murcia-Belmonte et al. 2010). This fact renders anosmin-1 as one of the first, to date, identified molecules that are involved in the migration of neuroblasts from the SVZ to the OB, before the rostral migratory stream has reached its mature conformation (García-González et al. 2010; Peretto et al. 2005; Petreanu and Álvarez-Buylla 2002). The arrival to the OB of newly generated neuroblasts from the SVZ is crucial for the maintenance of the olfactory function in rodents (Lois and Alvarez-Buylla 1994; Lois et al. 1996). Related to this, anosmin-1 has been shown to play a different role in glial cells: in oligodendrocyte precursors cells (OPC), the relative concentration of anosmin-1 and FGF-2 modulates OPC migration through their interaction with FGFR1 in the optic nerve during development and in the adult brain in mice (Bribián et al. 2006; Clemente et al. 2011). More specifically, anosmin-1 inhibits the motogenic effect of FGF-2 via FGFR1 (Bribián et al. 2006; Clemente et al. 2011). But this effect on migration is tightly related to cell adhesiveness and, in this cell type, anosmin-1 has a stronger adhesive effect than laminin and fibronectin, reducing cell motility in consequence (Bribián et al. 2006, 2008). In OPCs, anosmin-1 mechanism of action is FGFR1 independent and only due to interactions with other ECM proteins (laminin or fibronectin), including anosmin-1 itself in a homophilic way (Bribián et al. 2008). The OPC migration from oligodendrogenic sites to their final emplacement is fundamental for the correct myelination and function of the CNS (de Castro and Bribián 2005).

12.3.2 Axon Outgrowth and Collateral Formation

The first biological effect described for anosmin-1 was its role as a substrate promoting neurite growth in postnatal mouse cerebellar neurons (Soussi-Yanicostas et al. 1998). In that study, cerebellar neurons that co-culture with anosmin-1-expressing CHO cells showed a reduction in neurite growth and an induction in neurite fasciculation. The implication of anosmin-1 has also been found in other

CNS developmental processes in mammals. In rats, anosmin-1 finely attracts OB projection neuron axons (mitral and tufted cells) forming the LOT during a precise developmental time frame (Soussi-Yanicostas et al. 2002). Besides, anosmin-1 enhances collateral branching of LOT axons and exerts a chemoattractive effect on its collateral branches within the piriform cortex (Soussi-Yanicostas et al. 2002). Similar effects are observed in the cerebellum, where anosmin-1 promotes neuritic elongation and strongly increases the budding of collateral branches and the extension of terminal arbours in Pukinje cells from embryonic (E17) and newborn (P0) rats and in axonal regeneration after axotomy (Gianola et al. 2009). In human embryonic GnRH olfactory neuroblasts (FNC-B4 cells), anosmin-1 induces neurite outgrowth and cytoskeletal rearrangements through FGFR1-dependent mechanisms (González-Martínez et al. 2004).

12.4 Anosmin-1 Effects in Other Vertebrates

Two orthologs of the *KAL1* gene have been found in teleosts: *kal1a* and kal1b, encoding anosmin-1a and anosmin-1b, respectively. It has been demonstrated to be an essential requirement for anosmin-1a, but not for anosmin-1b, in GnRH cell migration in zebrafish and medaka (Okubo et al. 2006; Whitlock et al. 2005). In addition, while *kal1a* and *kal1b* display distinct transcription patterns during zebrafish development, both genes are strongly expressed in another migrating cell population from the posterior lateral line primordium (Ardouin et al. 2000), for which migration anosmin-1 is crucial (Yanicostas et al. 2008). More precisely, anosmin-1 seems to play a key role for proper activation of the CXCR4b/SDF1a and/or CXCR7/SDF1a signalling pathways (Yanicostas et al. 2008). Regarding olfactory system development in zebrafish, anosmin-1a depletion impairs the fasciculation of olfactory axons and their terminal targeting within the OB. In this sense, *kal1a* inactivation induces a severe decrease in the number of GABAergic and dopaminergic OB neurons (Yanicostas et al. 2009).

Related to anosmin-1/FGF-2/FGFR1 signalling, a recent study developed in chick embryos has described the effect of anosmin-1 on FGF-8 signalling (Endo et al. 2012). This work highlights the strong influence of anosmin-1 on three morphogen agents, belonging to some well-known families, such as FGF, BMP and WNT, known as key actors in the formation of the neural crest and craniofacial development (Sauka-Spengler and Bronner-Fraser 2008; Trainor et al. 2002) More specifically, Endo et al. (2012) show that anosmin-1 is synthesised locally in the neural crest microenvironment, up-regulating *FGF8* and *BMP5* gene expression. Anosmin-1 also enhances FGF8 activity, while inhibiting both BMP5 and WNT3a activities, being therefore crucial for the formation of cranial neural crest. This study supports the idea of the relevance of this ECM protein at temporally and spatially regulating growth factor activities during embryonic development (Endo et al. 2012) as we and others have suggested (See above).

12.4.1 Anosmin-1 Function in Invertebrates

In *C. elegans*, anosmin-1 ortholog is *kal-1*. In this nematode *kal-1* is involved in two important events in the epithelial morphogenesis: ventral enclosure and male tail formation (Rugarli et al. 2002). Thus, as well as in rodents, *kal-1* affects neurite outgrowth in vivo by modulating neurite branching (Rugarli et al. 2002) and also promotes migration of ventral neuroblasts prior to epidermal enclosure (Hudson et al. 2006). However, in this case, *kal-1* does not modulate FGF signalling in neuroblast migration but it seems that *kal-1* interacts with multiple HSPGs to promote cell migration.

12.5 Anosmin-1 in Kallmann Syndrome

KS is a genetically heterogeneous developmental disease, characterised by hypogonadotropic hypogonadism and anosmia, and its prevalence has roughly been estimated to be from 1:8,000 to 1:10,000 in men (Seminara et al. 1998) and around 1:40,000 in women (Dodé and Hardelin 2009). The presence of a defective sense of smell, whether partial (hyposmia) or complete (anosmia), distinguishes KS from normosmic idiopathic hypogonadotropic hypogonadism with a normal sense of smell (nIHH), which can be associated with mutations in the *GnRHR* and *GPR54* genes. Due to hypothalamic GnRH-1 deficiency, males with KS show cryptorchidism, testicular atrophy and microphallus at birth and then subsequent failure to undergo a normal puberty during adolescence. Females with KS usually present primary amenorrhea or infertility.

First described by the Spanish pathologist Aureliano Maestre de San Juan in 1856, KS is defined by the association of the presence of small testes (hypogonadotropic hypogonadism) with complete (anosmia) or incomplete (hyposmia) olfaction disturbance (Maestre de San Juan 1856). A more detailed description of the syndrome was reported almost a century later using patients from different affected pedigrees with hypogonadism and anosmia (Kallmann et al. 1944). They hint at a broader spectrum of clinical defects and identify the familial nature in the clinical syndrome that was seen in both sexes and accompanied by multiple congenital anomalies. In 1954, de Morsier first noted the link between hypogonadism and neuroanatomical defects, including agenesis of the olfactory bulb and tract and other midline neuroanatomical defects (de Morsier 1954). Since then, it is commonly accepted that hypogonadotropic hypogonadism in KS is caused by the migratory arrest of GnRH-1 neurons, failing to enter the telencephalon and lack of GnRH secretion leads to a complete or partial failure of pubertal development (Wray et al. 1989; Hayes et al. 1998; Schwanzel-Fukuda et al. 1989). However, in the case of hyposmia/anosmia a wide array of anatomical defects could be responsible for the lack of smell ranging from the agenesis/hypoplasia of the OBs to defects in the

formation of the olfactory nerve or the lateral olfactory tract and including other causes that may explain this sensory problem.

There are different modes of KS transmission described to date, that include X chromosome-linked recessive, autosomal recessive, autosomal dominant with incomplete penetrance, and most probably digenic/oligogenic inheritance [for more details, see Dodé and Hardelin (2009)]. Multiple genetic causes have been identified so far in the development of this disorder: *KAL1*, *FGFR1*, *PROK2*, *PROKR2*, *NELF*, *KISSR1*, *CHD7*, *SEMA3A* and *FGF8* (Young et al. 2012; Legouis et al. 1991; Dode et al. 2003, 2006; Kim et al. 2008; Pitteloud et al. 2007; Falardeau et al. 2008).

As described above, *KAL1*, the gene causing the X-linked form was the first to be identified in KS (Franco et al. 1991; Legouis et al. 1991). *KAL1* is localised in the Xp22.3 region and encodes anosmin-1, protein that shows a strong homology with adhesion molecules involved in neuronal migration and axonal pathfinding. As previously exposed in this work, *KAL1* has been extensively studied along the last decades, although the absence of an identifiable murine ortholog has denied researchers the opportunity to create and study *Kal1* knock-out mice (see above). Other gene related to KS is *KAL2*, responsible for the autosomal dominant variant of KS, which encodes FGFR1 (Dode et al. 2003). Numerous mutations on *FGFR1* have been described in several functional domains of this receptor. Genotype-phenotype correlations have shown that some clinical features associated with KS satellite symptoms, such as loss of nasal cartilage, hearing impairment, and anomalies of the limbs seem to be mainly associated with *KAL2* mutations. The role of FGFR1 in the normal development of the olfactory bulb explains the association of anosmia with GnRH-1 deficiency in FGFR1-mutated patients. Phenotype analysis indicates that FGFR1 is involved in normal migration of GnRH-1 foetal neurons, but this is clearly not the whole story as a substantial proportion of *KAL2*-mutated individuals have normosmic GnRH-1 deficiency (Martin et al. 2011).

Finally, recent works indicate that FGFR1 signalling is important for the generation of GnRH-1 neurons via the neurotrophic molecule FGF8 (Chung et al. 2010). Interestingly, FGFR1 is expressed by GnRH-1 cells (Gill et al. 2004), and FGFR1 hypomorphic animals show a dramatic reduction in the number of GnRH-1 neurons (Chung and Tsai 2010). Even more, FGF8 is involved in the induction and differentiation of the mouse nasal placode (Kawauchi et al. 2005), and the loss of this morphogen results in the absence of the vomeronasal organ and GnRH-1 neurons (Chung and Tsai 2010). Together with this, the region of the nasal placode from which the GnRH-1 cells emerge is missing in the homozygous FGF8 hypomorphs (Kawauchi et al. 2005). In this sense, recent findings have shown that anosmin-1 promotes the neural crest formation and controls, among other growth factors, FGF8 activity in chick embryo (Endo et al. 2012). However, it is noteworthy that since the entire region in the nasal placode is missing in these mice (homozygous FGF8 hypomorphs), the actual impact of these molecules on the development of GnRH-1 neurons and their precursors is not clear and therefore remains to be elucidated. All these observations together, focused on the description of FGFR1 and FGF8 mutations, shed light on the pathogenesis of GnRH-1 deficiency in general, not just KS (Martin et al. 2011; Villanueva and de Roux 2010).

12.6 Roles of Anosmin-1 in Other Diseases

12.6.1 Anosmin-1 in Multiple Sclerosis

Multiple sclerosis (MS) is the most frequent demyelinating disease in young adults. MS lesions are characterised by demyelination, inflammation, axonal loss and reactive gliosis (Frohman et al. 2006). Accompanying these events, a spontaneous, and sometimes extensive remyelination, is also possible under specific circumstances (Patani et al. 2007; Patrikios et al. 2006). However, in chronic MS lesions remyelination is absent or limited to the plaque border (Barkhof et al. 2003; Bramow et al. 2010; Prineas and Connell 1979). It is plausible that the permissive environment that allows the migration of OPCs during development should be present in this kind of demyelinating diseases, including MS, to produce an effective remyelination (Dubois-Dalcq et al. 2005). Nonetheless, the blockade of oligodendroglial progenitor differentiation is a major determinant of remyelination failure in chronic MS lesions (Kuhlmann et al. 2008). Associated to demyelinated plaques, alterations have been described in the expression pattern of several molecules involved in OPC biology during development such as CXCL1/GRO-α (Omari et al. 2005), semaphorin 3A and 3 F (Williams et al. 2007) and sonic hedgehog (Wang et al. 2008). Regarding the FGF2/anosmin-1 system, the distribution of FGF2 and anosmin-1 varies between the different kinds of demyelinated plaques in MS patients, showing a complementary spatial pattern (Clemente et al. 2011). In areas with active remyelinating activity, i.e. active lesions and the periplaque of chronic-active plaques (Frohman et al. 2006), FGF2 is up-regulated in infiltrating as well as microglia-derived macrophages, whereas anosmin-1 is absent (Clemente et al. 2011). In contrast, where the remyelination process is completely compromised i.e. demyelinated area of chronic-active and chronic-inactive plaques (Frohman et al. 2006), FGF2 is totally absent but Anosmin-1 appears filling the entire extension of both regions (Clemente et al. 2011). Although not ascribed to any cell type, anosmin-1 may be synthesised by astrocytes in these two particular regions, as it occurs in the cerebellum during development (Gianola et al. 2009). In addition, anosmin-1 is present in 13–14 % of the nude axons that pass through the demyelinated area but not in the periplaque or in its adjacent normal appearing white matter (Clemente et al. 2011). Therefore, up-regulated anosmin-1 during remyelination shows a similar profile to that found during human and other mammal development (Dellovade et al. 2003; Duke et al. 1995; Lutz et al. 1994; Soussi-Yanicostas et al. 2002). This is a striking functional histopathological observation and confirms that, in adult MS patients, axons acquire developmental features (Bribián et al. 2008; Soussi-Yanicostas et al. 2002). Similar re-expressions of ECM proteins in MS plaques have been previously shown. PSA-NCAM is re-expressed on 14 % of demyelinated axons in the plaques of chronic lesions but not in the periplaque or in the normal appearing white matter (Charles et al. 2002), and the glycosaminoglycan hyaluronan, as well as its binding transmembrane glycoprotein CD44, also accumulates in the core of chronic MS lesions (Back et al. 2005).

The up-regulation of different adhesion molecules in those regions where remyelination does not occur spontaneously could be interpreted in different ways. It may represent a part of a more general developmental programme reinitiated by neurons in order to protect themselves after the demyelinating injury. But it represents a non-desirable consequence since this neuroprotective activity possibly could render the axon non-permissive to remyelination. However, the mechanism by which adhesion molecules interfere with re-myelination is not well understood. A negative regulation of myelination by this kind of molecules could involve three different and hypothetical mechanisms (1) by triggering negative signals that impede oligodendrocyte maturation; (2) by steric inhibition, preventing a close contact between axons and oligodendrocytes; (3) by strengthening the adhesion of OPCs and inhibiting their migration. In the case of PSA-NCAM, it acts as a negative signal for myelination, probably by preventing adhesion of OPC processes to axons (Charles et al. 2000), but it also should be down-regulated to allow OPC differentiation (Decker et al. 2002). In this sense, PSA-NCAM expression persists in those regions containing unmyelinated fibres, such as the mossy fibres of the dentate gyrus and axons from the supraoptic and paraventricular nuclei, which remain unmyelinated throughout life (Seki and Arai 1991, 1993). During human foetal forebrain development, myelination starts in those areas where PSA-NCAM is down-regulated (Jakovcevski et al. 2007), which reinforces the data observed in mice. Hyaluronan also impedes remyelination in the corpus callosum by inhibiting OPC maturation when injected 5 days after lysolecithin-mediated demyelination (Back et al. 2005), but there are no data about its selective persistence in different tracts of the adult brain. The lack of data about anosmin-1 distribution in adulthood (of either human or other mammals) does not allow the consideration of similar roles of this molecule in adulthood or MS lesions. On the other hand, there are data suggesting that PSA-NCAM is not necessary for OPC motility but for the directional movement of OPCs in response to PDGF (Zhang et al. 2004), while anosmin-1 has been shown to antagonise FGF2 motogenic effect (Clemente et al. 2011; Bribián et al. 2006). Attending to developmental data about anosmin-1 actions on OPCs, two putative roles for axonal anosmin-1 in MS lesions could be considered (1) anosmin-1 may interfere with the FGF2 effects on OPC migration (Clemente et al. 2011; Bribián et al. 2006); (2) since homophilic interactions are important for axon-OPC recognition during development (Bribián et al. 2008), anosmin-1 may facilitate OPC recognition/adhesion and thereby facilitate remyelination.

Therefore, to mimic developmental conditions and induce effective remyelination, several aspects are needed in the FGF2/anosmin-1 system. First, FGF2 should be present and anosmin-1 absent in areas of successful remyelination (Clemente et al. 2011). Second, not only axons, but adult OPCs, should acquire embryonic characteristics (Clemente et al. 2011; Bribián et al. 2006, 2008), as it has been previously shown in MS for other OPC-specific genes (Arnett et al. 2004; Capello et al. 1997). Third, OPCs may express the fundamental receptor involved in anosmin-1 actions, FGFR1 (Hu et al. 2009; Murcia-Belmonte et al. 2010; Clemente et al. 2011). The first circumstance is present in areas with spontaneous remyelination (active lesions and the periplaque of chronic-active lesion). However, in the

demyelinated area of MS chronic lesions, FGF2 is totally absent and the lack of anosmin-1 in OPCs could affect their recognition of the nude anosmin-1-expressing axons and thus compromise their ability to repair the damage. Finally, a subpopulation of OPCs within areas where FGF2 is up-regulated expresses FGFR1, which suggests that FGFR1^{+}-OPCs are the cells recruited by this growth factor into these zones or it might up-regulate FGFR1 in OPCs, as observed previously in vitro (Bansal et al. 1996).

In the last years, a new concept about the relationship between the shape of the oligodendrocyte and the ECM content has emerged. Kippert et al. (2009) showed that the cell surface area of this cell type is critically dependent on actomyosin contractility and is regulated by physical properties of the supporting matrix. These authors also demonstrated that the presence of ECM proteins with known non-permissive growth properties within the CNS blocks oligodendrocyte surface spreading, which is accompanied by changes in the rate of endocytosis (Kippert et al. 2009). An implication of these findings would be that changes in the rigidity of the scarred MS lesion due to reactive astrocyte protein secretion may unbalance the intracellular and extracellular forces and inhibit oligodendrocyte differentiation. As has been described along this chapter, the core of MS chronic lesions presents an astrocyte-mediated deposition of different extracellular matrix proteins (anosmin-1, PSA-NCAM and hyaluronan). A first implication of their presence in this specific area could be an increase in the rigidity of the demyelinated area compared to the surrounded periplaque, and therefore, an unbalance in the cellular forces that drive the inhibition of remyelination. Another possibility unexplored to date is that, although these extracellular cues would not alter the physical properties of the demyelinated area, they would change the activity of signalling molecules that regulate intracellular force (specifically those related with RhoA) and could also inhibit remyelination (Baer et al. 2009; Bauer and Ffrench-Constant 2009). As described above, anosmin-1 is a firm candidate for this, since it induces cytoskeletal rearrangements through FGFR1-dependent mechanisms involving Cdc42/Rac1 activation, two members of the Rho family of small GTPases (González-Martínez et al. 2004). However, further experiments are needed to establish whether anosmin-1 also participates in controlling actomyosin contractility and, therefore, oligodendrocyte cell shape and differentiation.

12.6.2 Anosmin-1 in Other Pathologies

Besides their above-mentioned different roles in pathologies affecting the CNS (KS and MS), anosmin-1 has been also involved in totally different diseases. For example, anosmin-1 produced by epidermal keratinocytes in response to calcium concentrations or anti-inflammatory cytokines may modulate epidermal nerve density in atopic dermatitis (Tengara et al. 2010) and it has been also implicated in the response to immunoglobulin therapy in dermatomiositis (Raju and Dalakas 2005).

Finally, a relationship between anosmin-1 and cancer has also been reported. For instance, up-regulation of *KAL1* in breast tumour tissue compared to normal breast tissue has been described (Arikawa et al. 2011), while in a metastasising human oesophageal squamous cell carcinoma cell line, *KAL1* is down-regulated when compared to the non-metastasising parental line (Kawamata et al. 2003). Jian et al. (2009) demonstrated that *KAL1* gene expression plays an important role in cancer metastasis and protection from apoptosis of the tumoural cell (Jian et al. 2009). Screening of colon, lung and ovarian cancer cDNA panels indicated a significant decrease in *KAL1* expression in comparison to corresponding uninvolved tissues. However, *KAL1* expression increased with the progression of cancer from early stages (I and II) to later stages (III and IV) of the cancer, and a direct correlation between the TGF-β and *KAL1* expression in colon cancer cDNA has been reported. In colon cancer cell lines, TGF-β induces *KAL1* gene expression and secretion of anosmin-1 protein. Interestingly, hypoxia induces anosmin-1 expression that, in turn, protects cancer cells from apoptosis activated by hypoxia and increases cancer cell motility (Jian et al. 2009).

12.7 Concluding Remarks

The new roles of anosmin-1 in different biological processes, as well as in pathology, outline a scenario far beyond the olfactory system and Kallmann syndrome. Anosmin-1 plays relevant direct roles in cell adhesion, cell migration, axonal outgrowth and collateralisation, but the indirect effects of the protein, modulating FGF-signalling (via FGFR1), have been recently complemented by other morphogenic pathways like BMP and Wnt. Research has progressed significantly in the last years regarding anosmin-1: we have dissected the different domains of the protein which are relevant for its binding to the different known partners (FGFR1, laminin, fibronectin, anosmin-1 itself, HS, others), and it has been pointed as an important actor in the pathogenesis of MS, cancer metastasis and allergic processes. In this sense, anosmin-1 would be considered as one of the most actively studied ECM proteins, and its binding properties describe a panoply of biological effects which should be increasingly considered in development and in adulthood and in physiological and pathological scenarios, biasing the scientific community by the meaning of its name, undoubtedly linked to olfaction: nowadays, if a scientist smells anomin-1 in the air, she/he should consider this protein as a relevant actor in physiology and pathology, specially, in the nervous system.

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