The Pathogenesis of Radial Ray Deficiency in Thrombocytopenia-Absent Radius (TAR) Syndrome

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ABSTRACT

The genetic basis of thrombocytopenia-absent radius (TAR) syndrome was recently identified to be related to the RBM8A gene. The encoded protein (known as the Y14 protein) is widely expressed in human cells (including osteoblasts) and plays several essential intracellular functions. Hence, the pathogenesis of radial ray deficiency in thrombocytopenia-absent radius syndrome remains a mystery. The current paper reviews the pathogenesis of the clinical features of thrombocytopenia-absent radius syndrome and offers a hypothesis of pathogenesis through attenuation of the Fibroblast Growth Factor 8 signal in the mesoderm because of an increased degradation of the Fibroblast Growth Factor Receptor 1.

Key Words: Thrombocytopenia-absent radius. Syndrome. Ubiquitin. Fibroblast growth factor receptor 1.

INTRODUCTION

Thrombocytopenia-absent radius (TAR) syndrome is a rare syndrome in which there is thrombocytopenia and absence of the radius bilaterally. The thumb is usually preserved but it has several characteristic abnormalities. Other features of the syndrome include renal and cardiac defects, cow-milk allergy, and intellectual defects. The syndrome is inherited as autosomal recessive, and hence genetic counselling and discussion of risks in future children should be done with the parents. Prenatal diagnosis of TAR syndrome is also feasible.

Recently, the genetic basis of TAR syndrome has been identified to be related to the RBM8A gene. In about 95% of TAR patients, one copy of the RBM8A gene is not functional due to a null allele (because of 1q 21.1 deletion including the RBM8A gene); and the expression of the other copy is reduced as a result of non-coding SNPs in the 5'UTR or in the first intron (hypomorphic mutation). In the remaining 5% of TAR patients, bi-allelic mutations are found in the RBM8A gene: one inactivating (nonsense or frameshift) mutation, and one hypomorphic mutation.

From the developmental point of view, the pathogenesis of the radial ray deficiency of the TAR syndrome is a mystery for two main reasons. The RBM8A transcript is widely expressed in human cells including osteoblasts and osteocytes. Hence, the first question that arises is: Why do the developmental limb abnormalities only involve the radial ray of the upper limb bud? Secondly, preservation of the thumb is a characteristic feature of the TAR syndrome, which distinguishes it from other syndromes with longitudinal radial ray deficiency. The pathogenesis of this characteristic feature could not be explained in the literature.

The purpose of this article is to review the normal function of the RBM8A protein and the normal development of the radial ray including the relationship between the ectodermal Fibroblast Growth Factor 8 (FGF8) and its mesodermal Fibroblast Growth Factor Receptor 1 (FGFR1), and the relationship between FGFR1 and other clinical features in TAR syndrome such as thrombocytopenia, cardiac defects, cow-milk allergy, and intellectual defects.

Literature search strategy: The search was done using PubMed database. Two keywords were used: “Pathogenesis” and “TAR syndrome” with no time bar. A total of 108 articles were scanned and they were mainly explaining the genetic basis of the syndrome or describing the clinical features of the syndrome. None of the articles attempted to review or explain the pathogenesis of the clinical features of the syndrome. Hence, we decided to review the normal function of the RBM8A protein, the normal development of the radial ray, the ubiquitin system, and FGF8 within the developing limb bud. A hypothesis of the pathogenesis of the clinical features of TAR syndrome was then offered.

The normal function of the RBM8A protein: The protein RBM8A (RNA-binding motif protein 8A; also known as the Y14 protein) is one of the components of the exon-junction complex (Figure 1). The complex is deposited at splice junction on mRNA during pre-mRNA splicing and remains bound to mRNA until it is translated. The exon-junction complex has many cellular functions including the transport of mRNA from the nucleus to the cytoplasm, and the non-sense mediated...
mRNA decay. Non-sense mediated mRNA decay is part of the normal cell surveillance which degrades abnormal mRNA transcripts (mRNA that harbours premature translation-termination codon, and hence will result in synthesis of abnormal truncated proteins). Since RBM8A is widely expressed in human cells and considering the essential cellular functions of the exon-junction complex, complete loss of RBM8A is not compatible with life. However, isolated haplo-insufficiency (i.e. a single null allele) of RBM8A will not result in any abnormalities as evidenced by the apparently healthy carriers of the 1q21.1 deletion (i.e. with RBM8A haplo-insufficiency). As mentioned above, the TAR phenotype requires one non-functioning allele and a decreased expression of the other copy of the RBM8A gene.

The normal development of the radial ray of the upper limb bud: The upper limb bud (Figure 2) is composed of two main parts: the apical ectodermal ridge (AER) and the underlying mesoderm. The posterior mesoderm expresses sonic hedgehog (SHH), while the anterior mesoderm expresses SALL1 and SALL4 (Spalt-like proteins 1 and 4) as well as T-BOX5 (TBX5). The normal development of the ulnar ray (i.e. future ulna and fingers) requires normal SHH function/expression in the posterior mesoderm as well as normal SHH interactions with its overlying posterior part of the AER, which express Fibroblast Growth Factor 4 (FGF4); and the latter interaction is also known as the SHH-FGF4 loop. The normal development of the radial ray (future radius and thumb) requires normal SHH function and expression of SALL1, SALL4, and TBX5 in the anterior mesoderm as well as the normal expression of FGF8 in the anterior part of the AER. TBX5 is essential for the development of the radial ray through two different pathways (Figure 2). In the first pathway, TBX5 induces the expression of FGF10 in the mesoderm, and this will induce the expression of the wingless integrated protein WNT3A in the AER. WNT3A will then induce the expression of FGF8 in the AER and the latter maintains FGF10 in the mesoderm; and this is known as the FGF10-FGF8 loop. In the second pathway, TBX5 induces the expression of SALL4 which will form a heterodimer with SALL1. This will enhance the WNT canonical signalling, resulting in the enhancement of SALL4 expression.

The above knowledge of molecular biology helps explain the findings of experimental research that shows that diminished FGF8 function in the AER will lead to phenotypes that are remarkably similar to the classification spectrum of radial-ray deficiency. Similarly, mutations of TBX5 (Holt-Oram syndrome), SALL4 (Okihiro syndrome), and SALL1 (Townes-Brocks syndrome) will have overlapping clinical features including radial ray deficiency.

The ubiquitin/SUMO pathway: Protein regulation, control of protein / receptor degradation, and elimination of damaged proteins / DNA are the functions of several pathways/enzyme systems in the human body. One of these pathways is the ubiquitin/SUMO pathway. Ubiquitin or SUMO which are small polypeptides, conjugate with the substrate protein via conjugating enzymes. Another group of enzymes called ligases, provide substrate specificity through their substrate binding sites. This conjugation (by either ubiquitination or SUMOylation) will enhance substrate degradation. The conjugation process is reversible by de-conjugating enzymes. The relationship between the Y14 protein and ubiquitin: Within the cell, there are several regulated transport systems which transport molecules from the cytoplasm to the nucleus and vice versa. Importin 13 (Imp13) binds both the MAGO-Y14 complex and the
The relationship between FGFR1 and other clinical features in TAR syndrome: The TAR phenotype includes transient thrombocytopenia, cow-milk allergy, cardiac/renal defects, and intellectual disability. Thrombocytopenia is an essential feature of the TAR phenotype, and the level of Y14 is low in the platelets of TAR patients. The thrombocytopenia in TAR syndrome is secondary to low number of megakaryocytes. Furthermore, the low platelet count is known to improve as the child grows and may even normalise in adulthood. In infants and young children, the production of platelets and other blood cells occurs at a higher rate than older children and adults. Hence, the bone marrow hematopoietic cells are stressed during early life. Zhao et al. created a conditional FGFR1 knockout mouse model to study the relationship between FGFR1 and various hematopoietic stem and progenitor cells (HSPC) during both steady-states and stress conditions (the stress was induced by 5-fluorouracil treatment).

Normal megakaryocytes in steady-state had significantly higher FGFR1 than all other non-megakaryocytic cells. Strikingly, on day 5 after 5-fluorouracil treatment, FGFR1 levels increased 57-fold in megakaryocytes compared to only 16-fold in non-megakaryocytic cells. In steady-state, conditional knockout of FGFR1 did not significantly affect the phenotypical number of HSPC. Under stress, however, FGFR1 inactivation reduced the number of megakaryocytes (and not other HSPC). Our hypothesis correlates the reduction of the intracellular Y14 protein to the reduction of FGFR1 activity. The level of Y14 protein is known to be low in the platelets of TAR patients. Hence, the resulting low FGFR1 activity is expected to result in low megakaryocytes in the stressed bone marrow of infants and young children. The low FGFR1 in adults with steady-state bone marrow activity will not significantly affect megakaryocytes resulting in the recovery of thrombocytopenia.

Almost 50% of TAR patients have cow-milk allergy which is a form of atopy. Park et al. used direct sequencing to identify informative SNPs in the receptors of several candidate genes in a cohort of 2,055 children and adolescents. Atopy was significantly associated with haplotypes of FGFR1.

About 23% of TAR patients show cardiac defects and 15% of patients show renal defects. FGFR1 is required for cardiomyocyte differentiation and development as well as for nephron morphogenesis. Nguyen et al. reviewed the literature and found that 7% of TAR cases have intellectual disability. Furthermore, micro-deletions and micro-duplications of the 1q 21.2 region (which contains RBM8A) may present with isolated intellectual disability or schizophrenia without hallmarks of the TAR syndrome. FGFR1 signalling is not only critical to the proliferation and differentiation of neural progenitor cells, but is also associated with tyrosine hydroxylase activation in the brain. Schizophrenia is associated with abnormal dopamine activity in the brain and it is interesting to note that tyrosine hydroxylase is the rate-limiting enzyme in dopamine synthesis in the brain.

**DISCUSSION**

The relationship between radial ray deficiency in TAR syndrome and the suppression of Y14 protein in the cell is hard to explain not only because the Y14 protein is involved in several essential cellular functions, but also because 50% suppression of the normal levels of Y14 in the cell (i.e. with haplo-insufficiency of RBM8A) causes no abnormalities. The TAR phenotype requires greater than 50% suppression of the intracellular Y14. Since Imp13 binds to both the Y14 and the UbC9 in the cytoplasm, it is possible that beyond a certain critical low level of Y14, more ubiquitin will be available for FGFR1 degradation. When FGFR1 degradation reaches a critically high level, the FGF8 signal is attenuated enough to affect the skeletal development of the radial ray. As mentioned before, FGF8 is a diffusible protein from the AER. Hence, the concentration of FGF8 in the thumb area is higher than the area of the radius. Therefore, it is expected that critical attenuation of the FGF8 signal will be reached within the area of the radius.
before the thumb area. This may explain the most frequent observed phenotype of absent radius with preservation of the thumb.

If one accepts the above theory of pathogenesis via reaching critical levels, then one would also expect that some cases will also present with milder deformities (such as radial dysplasia rather than absence); and other cases will have severe functional attenuation of mesodermal FGF8 signals leading to failure of the entire limb to develop, as shown in experimental animals. Greenhalgh et al. studied the clinical features of 34 patients with TAR syndrome and found this variability of phenotypic expression even within the members of the same family.

The existence of a relationship between FGFR1 and other clinical features of TAR syndrome supports our hypothesis. The review identified the relationship between FGFR1 and transient thrombocytopenia and provided clear evidence from the literature that FGFR1 participates in the pathogenesis of cow-milk allergy, cardiac/renal defects, and intellectual disability.

CONCLUSION

Thumb preservation with absent radius in TAR patients may be explained by an enhanced mesodermal FGFR1 degradation beyond a critical level. This occurs despite the normal expression of FGF8 in the ectoderm and the normal expression of TBX5, SALL1, and SALL4 proteins in the mesoderm. Furthermore, our review of the literature revealed a direct relationship of FGFR1 signalling and other clinical features of the TAR phenotype. Although this is only a hypothesis, it may guide further research on the developmental biology of TAR syndrome.

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