Alteration of the Signal Transduction Pathway in RASopathies as a Basis of Targeted Therapeutic Drug Development

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Abstract: Ras/mitogen-activated protein kinase (MAPK) pathway is one of the most critical intracellular signalling cascades, relaying the extracellular signal in the form of growth factor into specific responses. The primary responses of Ras/MAPK pathway activation are cellular proliferation and differentiation. Therefore, a mutation in genes encoding one of its components or regulators causes a severe developmental disorder. RASopathy is a group of genetic syndromes originating from a germline mutation in the Ras/MAPK pathway’s regulators’ genes encoding components. More than 20 genes are associated with and seven syndromes included in RASopathy: Noonan, LEOPARD, neurofibromatosis type 1, CM-AVM, Costello, cardio-facio-cutaneous, and Legius syndrome. Genotype-phenotype associations in RASopathy are complicated, the mutation in one gene could result in different syndromes, while a mutation in different genes could cause one syndrome. Molecular diagnostic at the genomic level is crucial in establishing the definitive diagnosis and as the basis for targeted therapy. Several therapeutic agents target the MAPK pathway, but they have been mainly utilized in malignancy cases in which aberrant MAPK pathway was detected. Research in targeted therapeutic drug development in RASopathy is still limited, yet it is eminently needed for further elaboration.

1 INTRODUCTION

RASopathy is a group of syndromic genetic diseases due to germline mutation in genes encoding components or regulators of the Ras/mitogen-activated protein kinase (MAPK) signalling pathway. (Rauen, 2013; Romano et al., 2010) This pathway mediates the effects of growth factors and, consequently, plays an essential role in the growth of many cells and tissues. Ras is a GTPase protein encoded by the RAS gene that works following growth factor receptors activation, usually in tyrosine kinase receptor (TKR). The MAPK pathway is one of the most vital downstream signalling cascades of Ras. Regulatory nuclear proteins are the most common final target of this pathway most of which are transcription factors, histones, and other proteins having a role in the cell cycle, proliferation, differentiation, and cellular apoptosis and senescence. (Alberts et al., 2008; Morrison, 2012; Plotnikov, Zehorai, Procaccia, & Seger, 2011) Disruptions in the Ras/MAPK pathway will predictably result in a severe developmental disorder, either localized or systemic, such as what is found in RASopathy syndromes. Each type of RASopathy has distinctive characteristics, although there are still overlapping pathologies amongst them. Some common clinical manifestations observed in almost all RASopathy syndromes are abnormalities in the craniofacial region; malformation of the heart; skin, muscles, and ocular findings; neurologic and cognitive disorder; hypotonia; and a higher risk of developing malignancy. (Rauen, 2013)

The cumulative incidence of all RASopathy syndromes is 1 case in every 1000 live births. More than 20 mutated genes had been identified in RASopathies, and these genes encode proteins directly involved in the Ras/MAPK signalling

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pathway or their regulators. The relative position of these proteins in the pathway affects the degree of clinical severity: the more upstream its involvement in the pathway, the more severe the phenotypes. However, this correlation is not clear-cut as clinical heterogeneity is commonly observed in one specific RASopathy caused by q disruption in the same gene. (Rauen, 2013; Tajan, Paccoud, Branka, Edouard, & Yart, 2018) Several syndromes which belong to the RASopathy group are 1) Neurofibromatosis type 1 (NF1); 2) Noonan syndrome (NS); 3) NS with multiple lentigo or LEOPARD syndrome; 4) Capillary malformation-arteriovenous malformation (CM-AVM) syndrome; 5) Costello syndrome (CS); 6) Cardio-facio-cutaneous (CFC) syndrome; and 7) Legius syndrome. These syndromes overlap with each other in terms of clinical manifestations and their causal gene. On the other hand, one specific syndrome could also be caused by a mutation in different genes. This occurs partly due to the high degree of cross-linking in the MAPK pathway, and it has a potential clinical impact, especially for targeted therapy development. (Rauen, 2013; Wu-Chou et al., 2018)

As with other syndromic diseases, each RASopathy type is suspected based on the constellation of signs and symptoms. However, etiologic diagnosis to elucidate which gene is affected, and the type of the mutation affecting it is paramount. RASopathy syndromes caused by different genes or different mutation in the same gene might have distinct hereditary patterns and clinical courses. This information needs to be addressed in a genetic counselling session with the patients and their families. Moreover, to know the exact genetic defect is the first step in building the precise formula for personalized medicine through targeted therapy or even gene therapy; (Bai et al., 2019)

In this review, biomedical aspects of RASopathy, starting from the Ras/MAPK pathway's role in healthy to various genetic defects underlying each syndrome, and how it is related to the latest and future potential drug development in targeted therapy RASopathy were assessed.

2 RAS AND MAPK SIGNALING PATHWAY

The MAPK cascade comprises of four families, all of which are well-characterized, including classical MAPK known as ERK1/2, C-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK), p38 kinase, and ERK 5. (Belov & Mohammadi, 2012; Plotnikov et al., 2011) In each cascade three main kinases are sequentially activated, including MAPK kinase kinase (MAPKKK), MAPK kinase (MAPKK), and MAP kinase (MAPK). To this date, there are at least 17 MAPKKK, 8 MAPK, and 10 MAPK identified in mammalian cells. (Morrison, 2012; Zhang & Liu, 2002) As kinase is an enzyme catalyzing phosphorylation reaction, this sequential activation leads to phosphorylation of the regulatory proteins that are the signalling pathway's final target. This protein can be located in different subcellular locations, such as cytoplasm, mitochondria, Golgi apparatus, endoplasmic reticulum, and nucleus. However, the most common and important target protein in the MAPK signalling cascade is located in the nucleus and functions as gene expression regulators, whether it is a transcription factor, transcription activator/suppressor, or protein modulating chromatin remodelling. (Plotnikov et al., 2011)

2.1 ERK Pathway

The first and foremost identified MAPK pathway is the ERK1/2 cascade, and therefore it has been the benchmark for all other kinase cascades. This cascade has an important role in transducing extracellular signals mediated through various receptors, especially the RTK. Phosphorylation of receptor upon binding with its ligand provides a docking site for other proteins. Usually, the docked protein is an intermediary which promotes binding and subsequent interaction of other proteins in its vicinity. Such protein is known as an adaptor, and the most important adaptor in the MAPK pathway is growth factor receptor-bound protein 2 (Grb2). The Grb2 protein enables interaction between Ras protein and its activator, the sevenless (SOS) protein which functions as a guanine nucleotide exchange factor (GEF). Ras protein superfamily was named after the tissue and species from which it was first identified: Rat Sarcoma factor. It is found in the plasma membrane's cytoplasmic surface, anchored through a covalent bond to its lipid moiety. The protein has an intrinsic GTPase activity which hydrolyzes GTP into GDP so that the activated form has a very short half-life. Another protein serving as Ras regulator called GTPase-activating protein (GAP) promotes Ras GTPase activity. (Belov & Mohammadi, 2012; Nandan & Yang, 2011; Zenenos & Kyriianou, 2013)

The activated Ras protein has its GDP dissociated and prefers binding to GTP. This GTP-binding Ras can recruit and activate MAPKKK proteins such as Raf-1 and B-Raf. The exact mechanism for
MAPKKK activation has not been defined yet. However, either dimerization or phosphorylation may be involved in the process. The MAPKKK protein phosphorylates MAPKK, the MEK1/2 and it turns phosphorylates ERK1/2 as the last kinase tier, the MAPK. The phosphorylated ERK is translocated to the nucleus and can bind either transcription factor affecting gene expression or the DNA itself. The affected genes usually encode for proteins promoting cellular proliferation, differentiation and survival, and preventing apoptosis. (Morrison, 2012; Plotnikov et al., 2011)

2.2 JNK and p38 Pathway

These pathways are functionally different from the ERK pathway because they operate when intra- or extracellular stressors are present. Although both JNK and p38 pathway has its particular protein in each kinase tier, there is a substantial cross-talk between them. This cross-talk is kept in check by other proteins, the scaffold-like JNK-interacting proteins (JIP) so those specific substrates for each pathway are concentrated and well-compartmentalized.

Because the JNK pathway is the first to be known as responding to cellular stress, it is also named a stress-activated protein kinase (SAPK) pathway. There are several notable distinctions between the JNK and classical ERK pathway. First, its MAPKKK can be activated by proteins having an intrinsic GTPase activity other than Ras, such as Rac1 and CDC42. Second, the MAPKKK activation can also be achieved without those proteins' involvement but rather directly stimulated by an adaptor (e.g. TRAF).

Third, all proteins at the MAPK level belong to the JNK protein family, which has a threonine-proline-tyrosine (TPY) motif in their active domains. The JNK pathway is mainly detected in cells that respond rapidly to stress, such as neurons, cells of the immune system, and cells whose activity under the influence of insulin. (Morrison, 2012; Plotnikov et al., 2011; Zhang & Liu, 2002) The p38 pathway has its particularity: as a MAPK, the p38 protein can undergo autophosphorylation when it is near other molecules, for example, certain adaptor protein (Tab1 and ZAP-70) and an analogue of phosphatidylinositol. (Morrison, 2012; Plotnikov et al., 2011)

3 RASOPATHY SYNDROMES

Each syndromic disease classified as RASopathy results from a defect of at least one gene encoding signal transduction components in the MAPK pathway or its regulators. The relationship between the mutated gene and the resulting clinical syndrome is depicted in Figure 1 as a dashed blue line.

3.1 Noonan Syndrome

Noonan syndrome is a congenital genetic disease with a relatively high prevalence, the incidence of which is 1 in 1000 to 2500 live births. This syndrome has an autosomal dominant inheritance pattern with complete penetrance, but variable expressivity. Several clinical characteristics can be found in patients with NS, including the distinctive facial and musculoskeletal features which include a large skull with a narrow facial area, wide eyes, prominent epicant<hl>h</hl>al folds, ptosis, down-slanting palpebral fissure, low-set ear, as well as a short nose with a depressed nasal bridge; thoracic deformity; and congenital heart disease. (Romano et al., 2010)

Noonan syndrome is a type of RASopathy with the most heterogeneous genetic defects. The mutated gene and the type of mutation influence the phenotype. Mutations in PTPN11, encoding the enzyme tyrosine phosphatase, consistently show a significant association with the incidence of thoracic deformity, mild bleeding disorders, and distinctive facial features and stature. The nature of the mutation in that gene also influences the type of heart defect: missense mutations are associated with a higher likelihood of pulmonary stenosis and ASD, while a lower likelihood of cardiomyopathy. Meanwhile, mutations in SOS1, the gene coding for sons of sevenless (SOS) protein, have phenotypic characteristics of the integumentary system that overlap with CFC syndrome, and mutations in the SHOC2 gene are associated with different phenotypes (i.e. mitral valve disorders, growth hormone deficiency, hyperpigmented skin, ichthyosis, and developmental disorders). The Noonan syndrome caused by a KRAS mutation tends to have a more severe phenotype with more significant developmental and learning disorders.1 However, not a single phenotype is unique to either a specific gene or type of mutation. The illustrates the complexity of interactions between genes in the RAS / MAPK pathway. Other than the genes mentioned above, there are several other causative genes for Noonan syndrome (Table 1). However, there was no identified mutation in any genes associated with the RAS/MAPK pathway in a small number of cases. (Bai et al., 2019)
Table 1. Mutated genes and its prevalence in Noonan syndrome.

<table>
<thead>
<tr>
<th>No</th>
<th>Gene/protein</th>
<th>Subcellular effects</th>
<th>Characteristics of mutation</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PTPN11/SHP-2</td>
<td>Cytosolic phosphatase cutting phosphotyrosyl bond on activated RTK and indirectly activating SOS-activator</td>
<td>Conformational change causing catalytic site exposure $\rightarrow$ ↑ phosphatase $\rightarrow$ ↑ MAPK activation</td>
<td>42 (Bertola et al., 2006) 58.3 (Chinton et al., 2019) 60 (F. R. Lepri et al., 2014)</td>
</tr>
<tr>
<td>2</td>
<td>SOS1/Sos</td>
<td>As a Ras-activating GEF</td>
<td>Mutation in autoinhibition sites $\rightarrow$ ↑ GEF activity $\rightarrow$ ↑ RAS/MAPK pathway activation</td>
<td>11 (Cessans et al., 2016) 18 (F. Lepri et al., 2011) 20 (Roberts et al., 2007)</td>
</tr>
<tr>
<td>3</td>
<td>KRAS and NRAS/Ras</td>
<td>One of Ras subtypes which activates MAPKKK tier</td>
<td>KRAS: ↑ GDP/GTP dissociation rate $\rightarrow$ ↑ RAS GEF-independent RAS activation* (Carta et al., 2006)</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NRAS: de novo somatic mutation $\rightarrow$ ↑ GAP-resistant RAS activation (Cirstea et al., 2010)</td>
<td>1 (Cirstea et al., 2010)</td>
</tr>
<tr>
<td>4</td>
<td>RIT1/Ras subfamily</td>
<td>idem</td>
<td>$\uparrow$ ELK1 activity, a TF which activates MAPK and activated by the MAPK pathway(Aoki et al., 2013) (positive cycle feedback)</td>
<td>3.8 9 (Aoki et al., 2013)</td>
</tr>
<tr>
<td>5</td>
<td>RAF1 dan BRAF/Raf family</td>
<td>A type of MAPKKK</td>
<td>$\text{RAF1: } \uparrow$ Raf activity $\text{BRAF: } \uparrow$ Raf activity</td>
<td>15 (Kobayashi et al., 2010) 1.7</td>
</tr>
<tr>
<td>6</td>
<td>MAP2K1/MEK</td>
<td>idem</td>
<td>$\uparrow$ MEK kinase activity</td>
<td>4.2</td>
</tr>
</tbody>
</table>

*Based on structural analysis study digitally (Carta et al., 2006)

3.2 LEOPARD Syndrome

LEOPARD syndrome is also known as Noonan syndrome with multiple lentigos. The name "LEOPARD" is an acronym for its main manifestations: multiple Lentigos, Electrocardiographic abnormalities, Ocular hypertelorism, Pulmonary stenosis, Abnormal genitalia, developmental Retardation, and sensorineural Deafness. The prevalence of LEOPARD syndrome in the general population is unknown. However, it is thought to be significantly rare because to date, the total number of cases reported in the publications is no more than 300 patients. (Martinez-Quintana & Rodriguez-Gonzalez, 2012; Sarkozy, Digilio, & Dallapiccola, 2008) This syndrome is either has an autosomal dominant inheritance pattern with complete penetrance or occurred sporadically due to de novo germline mutation. The main clinical manifestations based on which the clinical diagnosis is made 1) characteristic facial features such as hypertelorism, ear malformations, and low ear with a folded helix; 2) hypertrophic cardiomyopathy; and 3) café-au-lait macules which are usually found on the face, neck and upper part of the torso. Also, several other signs and symptoms could be encountered in a person with LEOPARD syndrome, such as thoracic deformity, cryptorchidism, mild learning, and psychomotor skill development disorders, and malignancy in the form of juvenile myelomonocytic leukaemia. (Martinez-Quintana & Rodriguez-Gonzalez, 2012)

Although the Leopard syndrome is genetically heterogeneous, 95% of cases have mutations in one of the following genes: PTPN11, RAF1, and BRAF. However, as depicted in Table 1, these genes are the causative genes for more than 50% of NS cases, while mutations in BRAF are also found in a significant number of CFC syndrome cases. (Martinez-Quintana & Rodriguez-Gonzalez, 2012) (Table 2)
Table 2. Mutated genes and its prevalence in LEOPARD syndrome

<table>
<thead>
<tr>
<th>No</th>
<th>Gene/protein</th>
<th>Subcellular effects</th>
<th>Characteristics of mutation</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PTPN11/SHP-2</td>
<td>Cytosolic phosphatase cutting phosphotyrosyl bond on activated RTK and indirectly activating SOS-activator</td>
<td>Mutation inactive catalytic site $\rightarrow$ ↓ phosphatase $\rightarrow$ but ↑ MAPK activation</td>
<td>42 (Bertola et al., 2006) 58.3 (Chinton, Huckstadt, Moresco, Gravina, &amp; Obregon, 2019) 60 (F. R. Lepri et al., 2014)</td>
</tr>
<tr>
<td>2</td>
<td>RAF1/Raf family</td>
<td>A type of MAPKKK</td>
<td>↑ Raf activity</td>
<td>&lt;5-10 (Carcavilla et al., 2013)</td>
</tr>
<tr>
<td>3</td>
<td>BRAF/Raf family</td>
<td>idem</td>
<td>Limited ↑ Raf activity</td>
<td>&lt;1-5 (Carcavilla et al., 2013)</td>
</tr>
</tbody>
</table>

3.3 Neurofibromatosis Type 1

Neurofibromatosis-1 (NF-1) or Von Recklinghausen's disease is one of the RASopathy syndromes inherited with an autosomal dominant inheritance pattern. (Upadhyaya & Cooper, 2012) The prevalence of NF-1 reaches 1 every 3000 to 4000 individuals in the general population, based on various studies from Europe and America States. (Uusitalo et al., 2015) The diagnosis of NF-1 is made based on clinical manifestations. Some of the most commonly reported symptoms of NF-1 patients are café-au-lait macules, neurofibromas of the skin or oral mucosa, brownish (freckling) spot in the axillary region, Lisch nodules on the iris, bone abnormalities, and malignancy, particularly optic nerve gliomas, astrocytoma, and schwannoma. (Upadhyaya & Cooper, 2012) The cause of NF-1 is a mutation in the NF1 gene that encodes the neurofibromin protein. This protein functions as an activator of GTP-ase which works on Ras: GTP-ase hydrolyzes GTP bound to Ras for Ras to reinstate its inactive form. If there is either a reduction in its level of expression or its dysfunction, there will be overactivity of the Ras signalling pathway and subsequent cell growth and proliferation. (Bennett, Thomas, & Upadhyaya, 2009) Based on previous studies, 100% of patients with NF-1 have mutations in the NF1 gene even with heterogeneous types of mutations: it can be small deletions, missense or nonsense mutations, as well as splicing mutations. Mutations can accompany mutations in NF1 in other genes, either the ones which also play a role in the RAS/MAPK pathway such as PTPN11 and BRAF, or those which are not, such as P53. (Arima et al., 2017) Concurrent mutations in PTPN11 and NF1 genes may indicate a possibly different clinical entity known as Neurofibromatosis-Noonan Syndrome (NFNS). (Thiel et al., 2009; Wu-Chou et al., 2018) Meanwhile, additional mutations, particularly in other oncogenes (e.g. P53), increase a patient's probability with NF-1 developing a tumor. (Gottfried, Viskochil, & Couldwell, 2010)

3.4 Capillary Malformation-arteriovenous Malformation Syndrome

Capillary malformation-arteriovenous malformation (CM-AVM) syndrome is a RASopathy syndrome characterized by multifocal capillary malformations that are typically found on the face and extremities. This condition can also be accompanied by arteriovenous malformations (AVM) and arteriovenous fistulas. The AVM may occur in various tissues, including the skin, muscle, bone, and various internal organs, for instance, the heart and brain. Consequently, if the AVM ruptures and bleeding occurs, there will be life-threatening complications. In general, this syndrome is inherited with an autosomal dominant pattern. However, in 20-30% of cases, pathogenic mutations occur de novo.

322
Table 3. Mutated genes and its prevalence in CV-AVM syndrome

<table>
<thead>
<tr>
<th>No</th>
<th>Gene/protein</th>
<th>Subcellular effects</th>
<th>Characteristics of mutation</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>RASA1/RASA1</td>
<td>Ras inactivation</td>
<td>↓ GTPase activity $\Rightarrow$ ↑ Ras activity</td>
<td>50 (Amyere et al., 2017)</td>
</tr>
<tr>
<td>2</td>
<td>EPHB4/EphB4</td>
<td>RTK family which signals through MAPK pathway to exerts its effects</td>
<td>↑ MAPK pathway activation</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>Undetected</td>
<td>-</td>
<td>-</td>
<td>40</td>
</tr>
</tbody>
</table>

The mutated gene in CM-AVM syndrome can be either the RASA1 gene or the EPHB4 gene (Table 3). (Bayrak-Toydemir & Stevenson, 1993-2020) The RASA1 protein is a negative regulator of Ras through its activity as a GAP. (Kawasaki et al., 2014) Meanwhile, the EphB4 protein is a member of the RTK Eph family which plays a role in embryonic capillaries morphogenesis. Physiologically, one of the effects of binding between the EphB4 receptor and its ligand is the suppression of VEGF-initiated endothelial proliferation and migration through the RAS/MAPK signalling cascade. (Kim et al., 2002; Xiao et al., 2012)

3.6 Cardio-Facio-Cutaneous Syndrome

Cardio-facio-cutaneous (CFC) syndrome is characterized by prominent heart abnormalities (usually in the form of pulmonary stenosis with other valves' dysplasia, septal defects, hypertrophic cardiomyopathy, and arrhythmia), notable craniofacial characteristics, and concomitant skin abnormalities including xerosis, hyperkeratosis, ichthyosis, keratosis pilaris, dermatitis, melanocytic nevi, hemangioma, and palmpoplantar hyperkeratosis. Almost all patients with CFC syndrome suffered from neurological and cognitive disorders. Besides, some of them had significant abnormalities in one or several other organ systems: musculoskeletal, ocular, gastrointestinal, and endocrine systems. Because the clinical manifestations of CFC syndrome are very diverse, the definitive diagnosis is established when there is clinical suspicion and confirmed a pathogenic variant in one of the genes associated with the syndrome, that is the BRAF, MAP2K1/2, and KRAS genes. (Table 4) Mutations in these genes are predominantly de novo and have an autosomal dominant pattern of inheritance. (Rauen, 1993-2020)
Table 4. Mutated genes and its prevalence in CFC syndrome. (Rauen, 1993-2020)

<table>
<thead>
<tr>
<th>No</th>
<th>Gene/protein</th>
<th>Subcellular effects</th>
<th>Characteristics of mutation</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BRAF/BRAF</td>
<td>Activates MAPK (MEK1/2)</td>
<td>↑ MAPKK phosphorylation</td>
<td>75</td>
</tr>
<tr>
<td>2</td>
<td>MAP2K1/2/MEK1 dan 2</td>
<td>Activates MAPK</td>
<td>↑ MAPK phosphorylation</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>KRAS/KRAS</td>
<td>Ras subtype</td>
<td>↓ intrinsic GTPase activity → ↑ Ras activity</td>
<td>2-3</td>
</tr>
</tbody>
</table>

3.7 Legius Syndrome

The characteristic feature of Legius syndrome is multiple café au lait macules but without the presence of neurofibromas or any tumor manifestation as found in NF-1. Other clinical manifestations that can be encountered in patients with this syndrome are intertriginous freckles, lipomas, macrocephaly, and developmental or learning disorders. To date, only less than 500 individuals with Legius syndrome have been reported worldwide with confirmatory molecular diagnostic laboratory examinations. The inheritance pattern is autosomal dominant, and children born to individuals with Legius syndrome have a 50% chance of inheriting the pathogenic variant. The only gene associated with the incidence of this syndrome is the SPRED1 gene. The gene encodes the Spred1 protein, which physiologically functions as an inhibitor of the Raf1 kinase activity. The pathogenic variant of SPRED1 in Legius syndrome loses its physiological function resulting in continuous activation of the Raf1 kinase and increases downstream signalling from the MAPK pathway. A similar pathophysiological mechanism is found in NF-1 with NFI gene mutation, whose product’s function is very similar to Spred1 protein. This explains the substantial clinical overlap between Legius syndrome and NF-1. (Stevenson, Viskochil, & Mao, 1993)

4 POTENTIAL TARGETED THERAPEUTIC DRUG DEVELOPMENT FOR RASOPATHY

Despite the diverse genetic variations in RASopathies, the impact of gene mutations in all syndromes belonging to RASopathy cause an increase or activation of the RAS/MAPK pathway. Therefore, targeted therapy to the RAS/MAPK pathway is predicted to have a role in managing RASopathy (Figure 1, straight red lines). This targeted therapy focuses on the downstream part of the pathway to cover the full spectrum of pathophysiology in the RASopathy syndromes. Some of the MAPK pathway blockers include MEK inhibitors (MAPKK tier), BRAF and Raf inhibitors (MAPKKK tier), and an antagonist of RAS, the farnesyltransferase inhibitors. However, the clinical trials’ target population evaluating these drugs was cancer patients with proven mutations and dysregulations in the MAPK pathway.

Although some RASopathy syndromes are associated with an increased risk of developing malignancy, not all patients with RASopathy exhibit these clinical manifestations. This has resulted in excluding most patients with RASopathy syndromes from many clinical trials evaluating targeted therapy for the MAPK pathway. (Gross et al., 2020) Furthermore, most of the studies assessing the use of inhibitors against components of the MAPK pathway in RASopathy syndromes were preclinical studies with the primary objective of explaining disease's pathophysiological mechanisms, not determining the effectiveness or efficacy of the inhibitors. However, all trials reviewed in this article focused on evaluating the RAS/MAPK pathway inhibitors' effectiveness for RASopathy, although the majority of which were still in the preclinical phase.

Ascota et al studied lovastatin therapy in mice with NFI mutation, and it was observed that the attention deficit and spatial aspects of learning were improved. Lovastatin is a statin drug that inhibits the HMG-CoA reductase enzyme and acts as an inhibitor of Ras isoprenylation. This post-translational modification process is essential for normal Ras protein function. (Acosta et al., 2011) On the other hand, Lee et al used a different type of NS animal model in which the genetic basis of mutation was the overexpression of the PTPN11 allele. The mice harboring the mutation increased excitatory synapse function, deficits in long-term potentiation, and spatial learning. After the MEK inhibitors administration, all neurologic manifestations were alleviated. (Lee et al., 2014) Although not as prevalent as PTPN11 mutation, SOS1 mutation has
also been implicated in NS (Table 1), and an animal model bearing the mutations were either unviable or having severe cardiac hypertrophy. Chen et al showed that prenatal administration of MEK inhibitors to the mutated mice prevented embryonal lethality and the cardiac abnormalities that should have occurred otherwise. (Chen et al., 2010)

![Figure 1. RAS/MAPK pathway in RASopathy and potential targeted therapy. Its components and regulators implicated in RASopathy are shown in elliptical boxes, and the dashed blue lines represent the association between a component/regulator (if mutated) and each RASopathy syndrome. The red rectangle boxes are potential therapies, and the straight red lines connect them to their specific targets in RAS/MAPK pathway](image)

Other than NF-1 and NS, there is a relative scarcity of study on other RASopathy syndromes. A study by Inoue et al utilized transgenic mice with BRAF gain-of-function mutation which are most commonly seen in CFC syndrome, and it showed consistent phenotypes of embryonic skeletal abnormalities, lymphatic defects, heart defects, and liver necrosis. These mutations are lethal in the embryonic period. However, prenatal administration of MEK inhibitors causes early embryo development to return to normal. (Inoue et al., 2014) The Costello syndrome experimental animal model with gain-of-Function mutations in HRAS have been shown to have increased activation of the ERK pathway and cognitive deficits. In this study, the lovastatin effect on the syndrome was evaluated. Unlike in the NF1 and NS experimental animal models, its administration did not restore ERK signalling activity to baseline level, and the cognitive deficits persisted. There might have been pathophysiological differences underlying cognitive deficits among different syndromes and/or different mutations. (Schreiber et al., 2017)

Although their numbers are limited, several studies have demonstrated the pharmacological potential of MEK inhibitors in human subjects. Dombi et al. conducted a clinical trial on NF-1 patients with inoperable plexiform neurofibroma (PN). This benign tumor originates from the myelin sheath covering the nerves and causes chronic pain, physical disability, and impaired motor function. The MEK inhibitor selumetinib administration in this group of patients significantly reduced tumor size, reduced pain intensity, and improved motor function. This study is the first breakthrough to demonstrate the clinical usefulness of MAPK inhibitors in RASopathy syndrome and provides preliminary evidence as a basis for the treatment of other RASopathies (non-NF-1) using these substances. The United States Food and Drug Administration has granted permission to use selumetinib as a PN therapy in NF-1 patients. (Dombi et al., 2016)

For other RASopathies, as stated before, the clinical trials were still limited to animal subjects. Wu et al. used a mice model of NS with mutations in RAF1 and administered MEK inhibitor PD032590 during the prenatal period. The administration of this therapy prevented developmental disorders of the heart. (Wu et al., 2011) Even though it has not been approved for NS standard therapy, Andelfinger et al
applied the MEK inhibitor trametinib to two newborns with NS who developed heart failure due to hypertrophic cardiomyopathy. In both of these infants, myocardial hypertrophy experienced a significant improvement (partial reversal) within 4 months after the first therapy. (Andelfinger et al., 2019)

Clinical trials for MAPK-targeted therapy pathways that have been carried out in the context of cancer treatment show some substantial side effects and resistance that results from the initiation of negative feedback cycles. This can be a problem if these targeted therapies are used in RASopathy because the treatment will be continuous and given for a lifetime. However, the therapeutic doses required for RASopathy are predictably to be significantly lower than the doses required to produce cytotoxic effects in cancer cells. Hence, the likelihood of side effects is also lower. An essential consideration for RASopathy targeted therapy is that most RASopathies result from germline mutations, while most mutations in cancers are somatic.

Germline mutations underlying RASopathy cause a homeostatic burden in the form of continuous and stable tonic activation of the MAPK pathway since early in life. Consequently, there is a possibility of harmful regulatory mechanisms in each organ system as an adaptive attempt to restore homeostasis.

Targeted therapy for the MAPK pathway can be optimized if supplemented with therapy targeting these specific adaptation mechanisms. Another challenge in treating RASopathy using targeted therapy is that the specific mutation underlying each syndrome can modify sensitivity to MAPK pathway inhibitors. For example, several types of mutations in the PTPN11 gene in NS cause mutant SHP2 protein production that is resistant to the allosteric inhibitor of SHP2. Likewise, mutated RAF1 or MEK1 possibly encode mutant protein resistant to target inhibitor MEK therapy. (Gripp et al., 2020; Gross et al., 2020; Tajan et al., 2018)

5 CONCLUSION

RASopathies are a group of syndromic genetic disorders that result from germline mutations in genes encoding components or regulators of the Ras / MAPK signalling pathway. The syndromes included in RASopathy are Noonan syndrome, LEOPARD syndrome, neurofibromatosis type 1, CM-AVM syndrome, Costello syndrome, CFC syndrome, and Legius syndrome. Clinically, there are plenty of overlapping signs and symptoms among the syndromes so that diagnosis based solely on clinical manifestations is exceptionally challenging. On the other hand, except for Legius syndrome, genetic mutations underlying each syndrome are vastly overlapped to one and another. Therefore, targeted therapy, especially those that work in the downstream part of the RAS/MAPK signalling, could be a solution in the clinical management of RASopathy. All RASopathy syndromes have in common, both genotypically and phenotypically, the MAPK pathway's overactivity. However, treatment in the form of an inhibitor of the MAPK pathway has been more widely studied for cases of malignancies with signalling aberrations in this pathway. Thus, there is still an opportunity for research development in specific treatment targeting components of the MAPK pathway to treat RASopathy syndromes.

REFERENCES


Alteration of the Signal Transduction Pathway in RASopathies as a Basis of Targeted Therapeutic Drug Development


