RASopathies are a family of rare autosomal dominant disorders that affect the canonical Ras/MAPK signaling pathway and manifest as neurodevelopmental systemic syndromes, including Costello syndrome (CS). In this issue of the JCI, Dard et al. describe the molecular determinants of CS using a myriad of genetically modified models, including mice expressing HRAS p.G12S, patient-derived skin fibroblasts, hiPSC-derived human cardiomyocytes, an HRAS p.G12V zebrafish model, and human lentivirally induced fibroblasts overexpressing HRAS p.G12S or HRAS p.G12A. Mitochondrial proteostasis and oxidative phosphorylation were altered in CS, and inhibition of the AMPK signaling pathway mediated bioenergetic changes. Importantly, the pharmacological induction of this pathway restored cardiac function and reduced the developmental defects associated with CS. These findings identify a role for altered bioenergetics and provide insights into more effective treatment strategies for patients with RASopathies.
Mitochondria and the future of RASopathies: the emergence of bioenergetics

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RASopathies are a family of rare autosomal dominant disorders that affect the canonical Ras/MAPK signaling pathway and manifest as neurodevelopmental systemic syndromes, including Costello syndrome (CS). In this issue of the JCI, Dard et al. describe the molecular determinants of CS using a myriad of genetically modified models, including mice expressing HRAS p.G12S, patient-derived skin fibroblasts, hiPSC-derived human cardiomyocytes, an HRAS p.G12V zebrafish model, and human lentivirally induced fibroblasts overexpressing HRAS p.G12S or HRAS p.G12A. Mitochondrial proteostasis and oxidative phosphorylation were altered in CS, and inhibition of the AMPK signaling pathway mediated bioenergetic changes. Importantly, the pharmacological induction of this pathway restored cardiac function and reduced the developmental defects associated with CS. These findings identify a role for altered bioenergetics and provide insights into more effective treatment strategies for patients with RASopathies.

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present with increased birth weight, craniofacial abnormalities, and gastroesophageal reflux with oral aversion, which results in a failure to thrive. Individuals can also have skin anomalies, including excessive wrinkling, redundancy of skin over the hands and feet, deep plantar and palmar creases, and increased risk of benign or malignant tumors. Musculoskeletal abnormalities, such as hypotonia, are also reported. Most importantly, like many of the other RASopathies, the most detrimental aspect of CS is the development of severe cardiac hypertrophy. However, the molecular mechanisms linking HRAS activation with cardiac dysfunction remain unclear.

A recent translational study of 11 patients with CS showed bioenergetic anomalies, including a low fasting blood glucose, low levels of insulin-like growth factor-1, and elevated total cholesterol (6).
Moreover, patients had increased resting energy expenditure, indicating increased cellular basal metabolism (6). Similarly, HRAS p.G12S mice show mitochondrial fatty acid oxidation defects, hypoglycemia, poor weight gain, and cardiomyopathy (7).

In this issue of the JCI, Dard et al. suggest that bioenergetic alterations of the heart cause CS-associated hypertrophic cardiomyopathy (HCM) (5). Indeed, disruption of mitochondrial function is a recognized contributing factor in stress-induced heart disease and cardiomyopathy. The findings reveal disruption of mitochondrial proteostasis and defective oxidative phosphorylation in the hearts and skeletal muscle of CS mice (5). Importantly, the authors also uncovered a mechanism that implicates inhibition of AMP-activated protein kinase (AMPK) in CS. The observation that AMPK signaling is involved in mitochondrial turnover, proteostasis, and bioenergetics is not new; neonatal cardiomyocytes from AMPKα2-knockout mice show repressed expression of energy metabolism-related genes (8). However, Dard and colleagues described the role of AMPK in RASopathies (5), and its effects on mediating aberrant mitochondrial energetics in these disorders may reveal targets for treatment of HCM in patients with CM. Analysis of the master regulators, such as AMPK, on mitochondrial fatty acid oxidation might help identify what role HRAS mutations have in the bioenergetic process.

Bioenergetics

Cells metabolize glucose to carbon dioxide through the mitochondrial TCA cycle and through oxidative phosphorylation (OXPHOS), which is the most effective way to generate ATP (refs. 9, 10 and Figure 1, B and C). Relative amounts of ATP, ADP, AMP, and phosphocreatine/creatine modulate intracellular energy, while levels of glucose, fatty acids, and amino acids regulate nutrient status (9). In addition, calcium and ROS influence cellular energy metabolism (ref. 10 and Figure 1, B and C). Importantly, transcriptional regulation and activation of downstream RAS signaling pathways control metabolic sensors (10). One example involves the activation of the AMPKs. Once activated, AMPKs induce catabolic pathways needed to produce ATP, increase glycolysis, and enhance oxidation phosphorylation, through mitochondrial biogenesis, while simultaneously switching off the anaerobic pathways that consume ATP, such as protein, fatty acid, and cholesterol synthesis (9). Consequently, AMPKs serve as critical modulators of bioenergetics, targeting multiple genes involved in lipid, glucose, and protein metabolism, including peroxisome proliferator-activated receptor γ coactivator 1-α (PGC1α) as well as genes involved in cell growth, autophagy, polarity, transcription, and ion transport (11).

Bioenergetic dysfunction in Ras/MAPK signaling

Many symptoms associated with mitochondrial diseases are also present in RASopathies, especially those RASopathies that develop cardiomyopathies. Like the mitochondrial mutations that cause mitochondrial disorders, increased Ras/MAPK signaling can promote anaerobic glycolysis, glucose uptake, autophagy, lipid synthesis, and nucleotide synthesis through the pentose phosphate pathway (9). Increased pathway activation can also modulate mitochondrial respiration, although the mechanisms remain unclear. Therefore, it seems probable that gain-of-function mutations associated with increased Ras/MAPK signaling in RASopathies also alter bioenergetics, leading to increased mitochondrial dysfunction and worsened pathological outcomes. Dard et al. identified molecular abnormalities that affect mitochondrial homeostasis in CS (5). Previous studies in HRASG12V/−/− transgenic mice also identified effects in energy homeostasis in CS, with decreased fatty acid oxidation, altered metabolism of glucose, and impaired energy homeostasis (7).

Bioenergetic effects of Ras/MAPK activation have been extensively studied in cancer, where pathway activation is linked to increased turnover of mitochondria through enhanced mitophagy, modulating mitochondrial fission and dynamics (9). However, mitophagy alone cannot explain the mechanisms linking Ras and MAPK activation with OXPHOS; a concomitant increase in mitochondrial biogenesis is also likely required. ROS produced mitochondrially may play a role in cancer cell transformation by RAS or may mediate oncogene-induced cellular senescence (8). Moreover, HRAS p.G12V mutations can stimulate mitochondrial metabolism and increase the rate of oxygen consumption (12, 13). Finally, AMPK signaling has been associated with mitochondrial proteostasis (11, 14); its activation induces mitophagy and the selective degradation of mitochondria by autophagy and stimulates mitochondrial biogenesis through PGC1α (9). However, how any or all these processes directly tie into Ras/MAPK signaling and how they specifically affect RASopathies remains unclear (15).

AMPK dysfunction and mitochondrial respiratory chain defects are frequent causes of HCM and cardiomyogaly (8, 16–19). Dard et al. (5) showed that both mRNA and protein expression levels of AMPK were strongly inhibited in CS. Likely, the combination of aberrant signaling upstream of AMPK, disrupted localization of signaling effectors, and/or changes in cellular distribution contributed to the decreased AMPK activity observed in CS. The authors also suggested another explanation: a dysregulation in modulating microRNAs (miRs). Specifically, they identified an induction in miR-221*, which they suggest modulates and reduced AMPK expression and activity in CS cell models. Previous work established a potential role for miR-221* in Ras activation (20). Dard and authors also established an association between miR-221* and AMPKα2 expression; inhibiting miR-221* in CS models rescued cellular energy homeostasis (5). However, given that miRs often have multiple targets with differential expression between mice and humans, as well as other limitations, additional studies are required to fully understand how miR-221*, or other miRs, affects RASopathies and if the inhibition of miRs can reverse or treat patient phenotypes, including HCM, short stature, and/or the craniofacial anomalies found in RASopathies.

Bioenergetic dysfunction in other RASopathies

Mutations in mitochondrial genes in RASopathies suggest an overlap in mitochondrial biogenesis. Though not specifically evaluated by Dard et al. (5), mitochondrial dysfunction in RAS-related genetic disease manifests in observable mitochondrial defects, decreased adipogenesis, fat malabsorption, lymphatic...
disorders, increased energy expenditure, and enhanced insulin signaling (9). In NS, mutations in PTPN11, the gene encoding the protein tyrosine phosphatase SHP2, lower mitochondrial membrane potential and ATP cellular content and increase ROS levels (21). Increased SHP2 activity has also been associated with increased complex IV activity and decreased ATP synthesis, altering cellular energy homeostasis (22, 23). Conversely, a BRAF-associated NSML mutation had decreased complex I activity (22). In NF1, data are more controversial; whereas decreased mitochondrial respiration, mediated by inhibition of respiratory chain complex II was noted in cells lacking NF1, increased mitochondrial respiration was observed in Drosophila lacking NF1 (24). Previous studies in CFC Syndrome also indicate mitochondrial dysfunction, with multiple enzyme deficiencies in OXPHOS, reduced ATP production, and a deficiency in muscular coenzyme Q, an enzyme required for transport of electrons from complexes I and II to complex III (22).

Factors contributing to mitochondrial dysfunction in RASopathies

Germline or somatic HRASG12S/A mutations can alter mitochondrial proteome homeostasis and OXPHOS in a tissue-specific manner (5). Indeed, at the level of the respiratory chain, tissues exhibit large differences in how they compose OXPHOS machinery (10). Heterogeneity of mitochondrial function within different cells means changes in cellular and mitochondrial microenvironments may regulate energy production. In CS, these differences in tissue expression can affect craniofacial development, heart structure and function, skin, and predisposition to cancer (25). Differences in the way RASopathy mutations interact with downstream effectors of the Ras/MAPK pathway also affect tissue function to mediate disease severity. However, how tissue specificity, localization, crosstalk, and/or degree of expressivity in various cell types and tissues contribute to mitochondrial dysfunction and bioenergetics in RASopathies remains unknown. Dard et al. suggest that AMPK inhibition alone cannot sufficiently induce heart dysfunction; additional stressors, such as dysregulated signaling through the HRAS/MAPK pathway or another challenge in mitochondrial bioenergetics, must occur (5).

Potential therapeutic approaches and targeted treatments

The pathophysiology of most RASopathies includes systemic alterations of energy metabolism, but the molecular mechanisms associated with this dysfunction were previously unknown. Therefore, the therapeutic implications of this study are great, in that they broaden the scope of the therapeutic potential for treating patients with RASopathies. First, metabolic remodeling and hormonal control of glucose and lipid catabolism can modulate bioenergetic regulation (26). Therefore, drugs that drive energy metabolism (e.g., AICAR, bezafibrate, resveratrol, cannabinoids, vitamins, etc.) could be considered as modulators of bioenergetics in RASopathies (27). Here, too, Dard et al. identified a benefit in treating CS with a downstream effector of AMPK, bezafibrate, an agonist of PPARα (ref. 28). The authors rescued or prevented defective mitochondrial dysfunction, improved heart function, and increased survival in various CS models (5). Moreover, they went on to show that a combination of bezafibrate with ursodeoxycholic acid in a natural compound produced by gut bacteria (29, 30), provided a synergistic effect, resisting the genetic developmental defects in the CS zebrafish model and preventing the HCM phenotype in the CS mouse (5).

Clinical trials for use of Ras and MAPK inhibitors in RASopathies are ongoing, but the results appear promising (31). Combining these inhibitors, perhaps at lower concentrations to prevent side effects, with bioenergetics compounds could prove fruitful. The findings by Dard et al. (5) provide optimism that targeted and effective treatments for RASopathies are on the horizon.

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