Chapter 9

Intracellular Pathways Associated with the Etiology of Autism

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Additional information is available at the end of the chapter

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Abstract

This chapter explores the relationship between the genes and proteins of the Akt and MAPK pathways and autism. This chapter presents the biology of these two pathways, their genes and cascading proteins, and then, it looks at the research that has connected these molecules to autism. Finally, it imparts current and future therapeutic modalities that might exploit abnormalities in these genes and proteins, change them and ultimately alter aberrant autistic behaviors.

Keywords: autism, Akt, MAPK, ERK

1. Introduction

In this chapter, we will review (1) the biology of intracellular pathways, with particular emphasis on the Akt and the MAPK pathways, (2) the potential association of Akt and MAPK pathway markers and autism, and (3) current and potential therapy as it might be related to these pathways.

This chapter is not a review of all pathways and biomarkers in cells, but, instead, the focus is on two prominent pathways, the Akt and MAPK, known to play a role in the etiology of autism.

2. The biology of the Akt and MAPK intracellular pathways

2.1. Structure and function of intracellular pathways associated with autism

2.1.1. Receptors and ligands

Intracellular pathways are initiated by ligands, such as growth factors, which attach to receptor proteins embedded in cell membranes (**Figure 1**). The stimulation of a pathway receptor initiates the pathway signaling that ultimately results in changes in DNA transcription and/or translation, and effects processes such as cell division, protein production, cell morphology and motility.

Two receptor proteins [epidermal growth factor receptor (EGFR) and c-Met (also called MET or hepatocyte growth factor receptor)], and their respective ligands [epidermal growth factor (EGF) and hepatocyte growth factor (HGF)] (**Figure 1**), will be reviewed here. Both of these receptor/ligand pairs have been found to initiate the Akt and MAPK pathways and have been implicated in the etiology of autism.

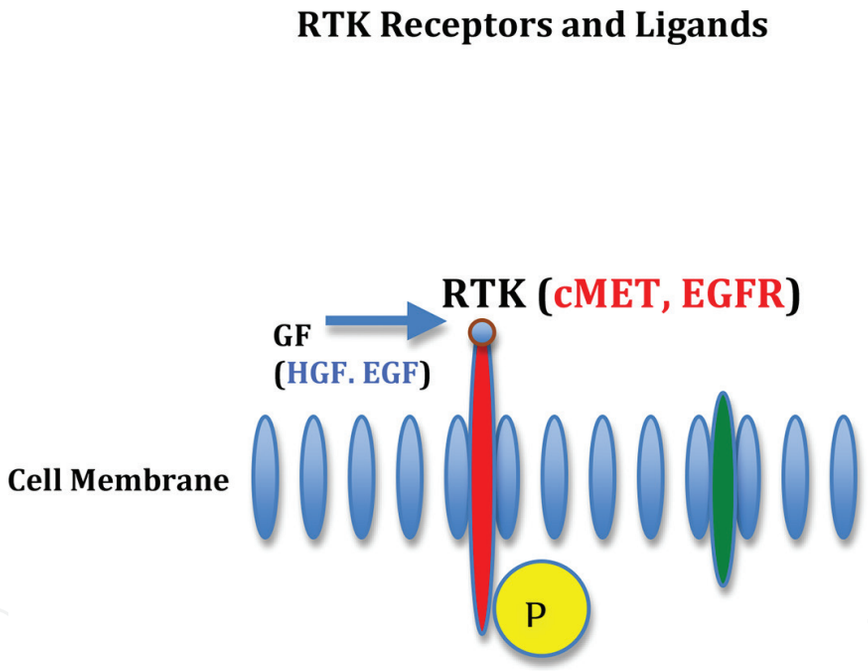

Figure 1. Membrane receptor [receptor tyrosine kinase (RTK)], either cMET or epidermal growth factor (EGFR) is signaled by growth factor (GF), either HGF or EGF, respectively. The interaction leads to phosphorylation inside the cell.

2.1.2. EGFR and EGF

EGFR is a glycoprotein that is a member of the protein kinase superfamily [1], and therefore is one of the receptor tyrosine kinases (RTKs). Binding of the receptor protein to the ligand, EGF, induces receptor dimerization, which stimulates its intracellular protein tyrosine kinase activity. As a result, there is autophosphorylation of several tyrosine (Y) residues in the C-terminal domain of EGFR [2]. This autophosphorylation causes downstream activation and signaling by several other proteins that react with the phosphorylated tyrosines.

The downstream signaling proteins initiate several signal cascades or pathways, principally the MAPK and Akt pathways [3]. These activated pathway proteins may lead to DNA synthesis, cell proliferation, cell migration, adhesion and proliferation [3].

Human EGF is a small 6045-Da protein [4] with 53 amino acid residues and three intramolecular disulfide bonds [5]. EGF acts by binding with high affinity to epidermal growth factor receptor (EGFR) on the cell surface. This stimulates ligand-induced receptor dimerization [6].

2.1.3. cMET and HGF

c-Met, or MET, is a membrane receptor protein that also possesses tyrosine kinase activity [7] and, therefore, is also a RTK. This cell surface receptor is expressed in epithelial cells of many organs, including the liver, pancreas, prostate, kidney, muscle and bone marrow, during both embryogenesis and adulthood [8]. c-MET has been shown to interact directly with the epidermal growth factor receptor (EGFR), allowing activation of c-MET after stimulation of cells with EGF [9, 10].

The ligand for c-MET is hepatocyte growth factor (HGF) [11, 12]. It is secreted as a single inactive polypeptide and is cleaved by serine proteases into a 69-kDa alpha-chain and a 34-kDa beta-chain. A disulfide bond between the alpha and beta chains produces the active, heterodimeric molecule [13].

HGF, through its signaling of c-MET, has been found to regulate cell growth, cell motility and morphogenesis through its activation of the c-Met receptor [14].

2.1.4. Downstream MAPK and AKT pathways

Stimulation of RTKs by EGF or HGF begins the downstream cascade of activated pathway proteins. The cascade starts when a signaling molecule (i.e., EGF or HGF) binds to the RTK on the cell surface. The pathways are chains of proteins that activate each other in a cascade fashion. A protein in the pathway is inactive, until it is phosphorylated by another protein in the cascade. Once it is activated, it can then catalyze the next substrate (protein) in the pathway. Overall, the proteins communicate a signal from the receptor on the membrane to the DNA in the nucleus. The DNA, through protein translation, orchestrates a change in the cell, such as increasing or decreasing cell division, cell morphology or cell motility.

2.1.5. Downstream MAPK pathway

The mitogen-activated protein kinases (MAPK) pathway (**Figure 2**) may begin with binding of EGF to EGFR. This activates the tyrosine kinase activity of the portion of the receptor in the cytoplasm. Docking proteins such as GRB2 (growth factor receptor-bound protein 2) contain an SH2 domain that binds to the phosphotyrosine residues of the activated receptor [15]. GRB2 then binds to the guanine nucleotide exchange factor Son of Sevenless (SOS) by way of the two SH3 domains of GRB2. When the GRB2-SOS complex docks to phosphorylated EGFR, SOS becomes activated [16]. (SOS was inactivated until it binds to activated EGFR, hence the cascade begins.) Activated SOS then promotes the removal of GDP from a member of the Ras subfamily (most notably H-Ras (transforming protein p21) or K-Ras (V-Ki-ras2 Kirsten rat

sarcoma viral oncogene homolog). Ras can then bind guanosine-5′-triphosphate (GTP) and become active. Activated Ras activates the protein kinase activity of RAF (proto-oncogene serine/threonine-protein kinase) kinase [17]. RAK kinase then phosphorylates MEK1 and MEK2 (mitogen-activated protein kinase kinase). MEK then phosphorylates and activates mitogen-activated protein kinase (MAPK), also known as extracellular signal-regulated kinases (ERK).

MAPK regulates the activities of several transcription factors. It can phosphorylate factor MYK, which plays a role in cell cycle progression, apoptosis and cellular transformation [18], and it can phosphorylate and activate MNK (interacting protein kinases), which, in turn, phosphorylates cAMP response element-binding protein (CREB). CREB has a well-documented role in neuronal plasticity and long-term memory [19] (**Figure 2**).

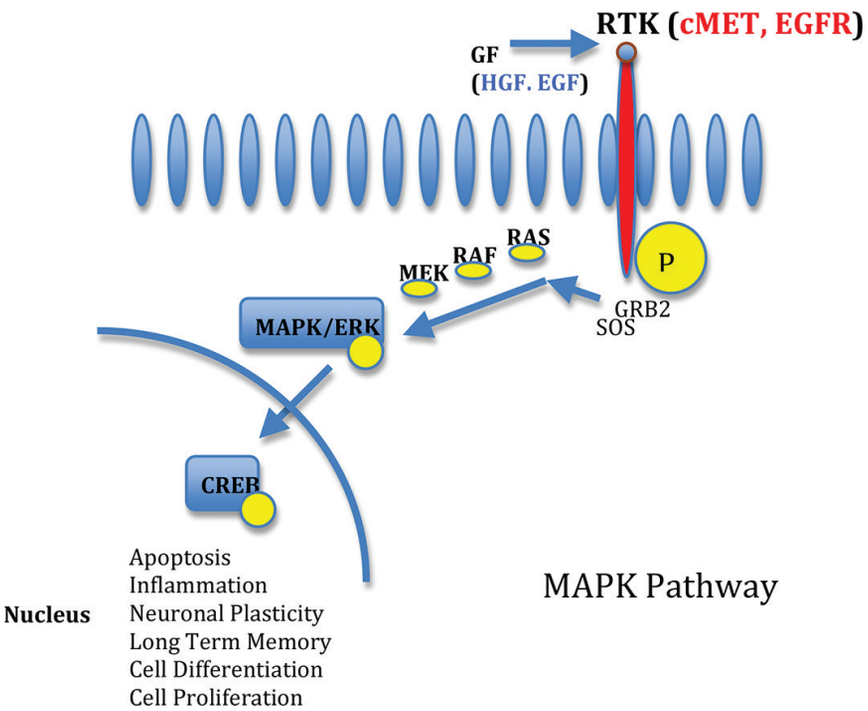

Figure 2. The MAPK pathway may begin with a growth factor (EGF or HGF) binding to the membrane RTK (cMET or EGFR). A cascade of activation ensues when the cytoplasmic portion of the RTK is phosphorylated, which, in turn, reacts with GRB2. At the end of the cascade, a transcription such as CREB affects protein production.

2.1.6. Downstream AKT pathway

The Akt pathway also starts with stimulation by a growth factor (i.e., EGF). This causes activation of a cell surface receptor (i.e., EGFR) and phosphorylation of phosphatidylinositol 3-kinase (PI3K) (**Figure 3**). Activated PI3K then phosphorylates lipids on the plasma membrane, forming second messenger PIP_3 (phosphatidylinositol (3,4,5)-trisphosphate). Akt, a serine/threonine kinase, is recruited to the membrane by interaction with these docking sites, so that it can be fully activated [20] (**Figure 3**).

Activated Akt causes downstream activation, which, by phosphorylating a range of intracellular proteins, ultimately results in changes in cell survival, growth, proliferation, cell migration and/or angiogenesis.

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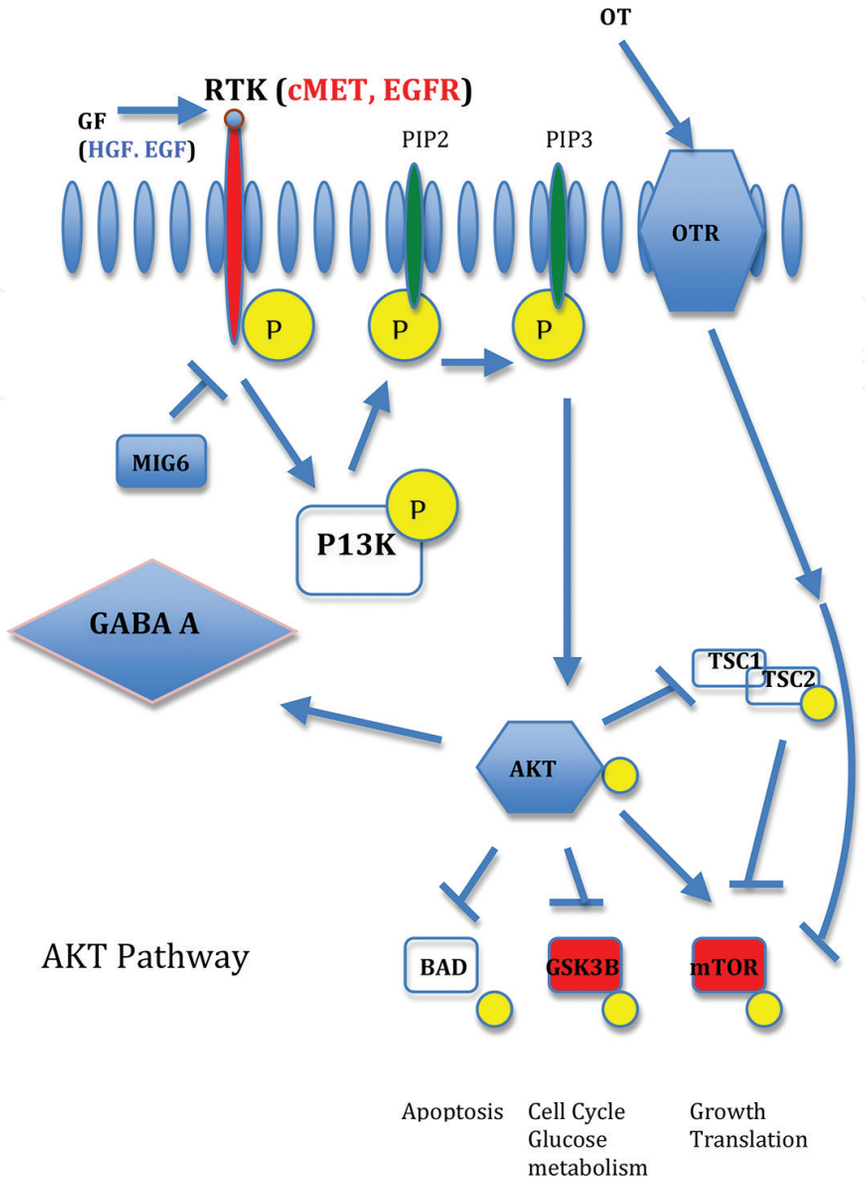

Figure 3. The Akt pathway may begin with a growth factor (EGF or HGF) binding to the membrane RTK (cMET or EGFR). A cascade of activation ensues when the cytoplasmic portion of the RTK is phosphorylated, which, in turn, reacts with P13K. At the end of the cascade, pathway proteins such as mTOR and GSK3 are affected.

The downstream effects of the Akt pathway are thoroughly regulated. The pathway is negatively regulated by phosphatase and tensin homolog (PTEN), which antagonizes PI3K. Demonstrated by the fact that loss of PTEN function leads to over-activation of Akt [21], the Akt pathway, in turn, regulates PTEN levels by activating transcription factors such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). These factors activate PTEN agonists, such as tumor necrosis factor alpha (TNFα), which, in turn, suppress PTEN [22].

Once it is activated, Akt interacts with its many substrates. As examples, Akt can phosphorylate Bcl-2-associated death promoter (BAD) causing it to lose its pro-apoptotic function, and promote cell survival [23]. Under various circumstances, activation of Akt has been shown to overcome cell cycle arrest in the G1 [24] and G2 [25] phases. Once activated, it can also affect cell division by

activating the transcription factor CREB [26], inhibiting the enzyme inhibitor p27 (cyclin-dependent kinase inhibitor 1B) [27], localizing the transcription factor, forkhead box class O (FOXO) in the cytoplasm [27], activating the membrane lipid, phosphatidylinositol (PtdIns)-3ps [28], and activating the kinase mechanistic target of rapamycin (mTOR) [25], which can affect transcription of p70 or eukaryotic initiation factor 4E binding protein 1 (4EBP1) [27]. Glycogen synthase kinase 3 (GSK-3) is inhibited upon phosphorylation by Akt. This results in an increase in glycogen synthesis [29]. Akt also enhances pathological angiogenesis and tumor growth associated with abnormalities in skin and blood vessels [25, 30].

2.2. Other biomarkers related to MAPK or Akt pathways

2.2.1. Oxytocin

There is evidence suggesting that oxytocin (OXT) is a regulator of the PI3K/Akt/mTORC1 pathway in gut cells and may, therefore, modulate translation in these cells [31].

The MAPK pathway is thought to be upregulated in the paraventricular (PVN) nuclei during lactation, and many of OXT's effects in peripheral and brain tissue are mediated through a MAPK/ERK pathway. Data also suggest an enhanced activation of the MAPK/ERK pathway in OXT neurons, specifically during late pregnancy in both the SON and PVN [32].

2.2.2. GABA

There is also evidence suggesting a relationship between the Akt pathway and GABA transmission. This relationship may be important in glucose metabolism and B cell proliferation. In human islets, GABA activates a calcium-dependent signaling pathway through both GABA-A and GABA-B receptors. This, in turn, activates the P13K-Akt and CREB-IRS-2 signaling pathways that convey GABA signals responsible for β-cell proliferation and survival [33].

The MAPK pathway may be a negative modulator of GABA-A receptor function [34]. Also, baicalin, a flavone glycoside, which modulates MAPK, has been found to have neuroprotective properties as a sedative associated with altering GABAergic signaling [35].

3. Association between pathway genes, proteins and autism

3.1. Genes associated with autism

Twin studies suggest that autism is highly heritable [36, 37], but there has not been just one gene associated with the etiology of the disorder(s). This could be because the majority of genes linked to autism are related to a specific symptom. Instead, not one, but many genes that are known to play a role in brain development are candidates for conferring susceptibility to autism.

According to the Simons Foundation Autism Research Initiative (SFARI) (Updated June, 2016), there are more than 800 genes implicated in autism, with annotations and links to published papers [38, 39]. The SAFARI gene-scoring module offers critical evaluation of the

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strength of the evidence for each gene's association with autism, with at least 50 high-ranking candidate risk genes so far identified.

We will be focusing here on only a few of the high-ranking autism-associated gene candidates, with particular emphasis on genes related to the Akt and MAPK pathways.

3.2. Pathway genes

MET (met proto-oncogene) encodes the hepatocyte growth factor receptor. MET, which has tyrosine kinase activity. Positive associations between MET and autism have been found in the Caucasian, Japanese and Italian populations as well as in Autism Genetic Resource Exchange (AGRE) family cohorts from multiple studies [40–42]. Interestingly, a positive association has also been found between MET and schizophrenia [43]. There is evidence to suggest that MET variances and MET/AKT interaction may affect facial emotion perception, implicating that the MET/AKT cascade plays a significant role in facial emotion perception [44].

Phosphatase and tensin homolog (PTEN) gene encodes a phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase, which contains a tensin-like domain, as well as a catalytic domain similar to that of the dual specificity protein tyrosine phosphatases. Several studies have found rare single variations in the PTEN gene associated with autism [45–47], including in Cowden syndrome [48], where some individuals with the syndrome develop autism.

Engrailed homeobox 2 (EN2) is a homeobox gene that is critical to the development of the midbrain and cerebellum [49]. It regulates the combined processes of cell division, differentiation and organ development. Four candidate gene studies have explored the relationship between EN2 and autism—three found a positive association.

Reelin (RELN) gene produces an extracellular matrix protein with serine protease activity [50], which is responsible for correct lamination of the brain during the embryonic period and cell signaling and synaptic plasticity in adult life [51]. Deficits in brain levels of Reelin mRNA and protein have been found in subjects with schizophrenia and bipolar disorder [52], as well as major depression [53] and autism [54]. Genetic studies show an association of polymorphisms in the RELN gene in autism [55, 56]. Reelin has recently been shown to activate Akt, as well as Src family kinases, suggesting a relationship between Reelin and the Akt pathway [57].

GABA-A receptor, BETA-3 polypeptide (GABRB3) encodes the GABA receptor. GABA is the main inhibitory neurotransmitter in the adult brain. During neurotransmission, GABA activates the GABA-A receptor. Data suggest that rare variants of GABRB3 might be associated with autism, and increased GABRB3 expression may contribute to the pathogenesis of autism in some patients [58]. Insulin-induced translocation of GABA-ARs to the cell surface requires activation of Akt, a primary target of insulin signaling downstream of PI3K. Akt phosphorylates a conserved phosphorylation site present in all three b subunits of GABA-ARs, which increases cell surface expression of these receptors [59, 60].

Fragile X mental retardation 1 (FMR1) mutations are associated with a substantial degree of increased risk of autism and are consistently associated with additional features not required for an ASD diagnosis [61]. The fragile X mental retardation protein (FMRP), the gene product

of FMR1, is an RNA-binding protein that negatively regulates translation in neurons. The Akt pathway is affected by this protein, which is demonstrated by the data that show that mTOR phosphorylation and activity are elevated in the hippocampus of juvenile FMR1 knockout mice [62].

Neurfibromin 1 (NF1): Genetic association has been found between the NF1 gene and autism. In particular, positive association was found with a NF1 polymorphism in the Japanese population [63]. The NF1-encoded protein, neurofibromin, functions as a Ras-GTPase activating protein, and the Akt/mTOR pathway is tightly regulated by neurofibromin [64]. Neurofibromatosis type 1 (NF1) is an autosomal dominant neurocutaneous syndrome, resulting from functional inactivation of the NF1 gene. Identification of SPRED1 gene mutations in individuals with mild NF1-like syndrome, and the direct involvement of these proteins in the RAS-MAPK pathway, potentially links the action of the NF1 gene to this pathway [65].

Tuberous sclerosis 1 (TSC1): This gene has been associated with syndromic autism, where a subpopulation of individuals with tuberous sclerosis syndrome develop autism [66]. This gene encodes a growth inhibitory protein thought to play a role in the stabilization of tuberin, and the protein has been implicated as a tumor suppressor. Akt and protein kinase B (PKB) both regulate and are regulated by the TSC1-TSC2 complex by interacting with and regulating mTOR [67].

Tuberous sclerosis 2 (TSC2): In addition to this gene's association with TSC1, its association with autism has also been found in an AGRE cohort [68], and a rare mutation in TSC2 has been identified in an individual with ASD [69].

Protein tyrosine kinase 7 (PTK7) is a gene strongly enriched for variants likely to affect ASD risk [70]. This gene encodes a member of the receptor protein tyrosine kinase family of proteins that transduce extracellular signals across the cell membrane. This protein may be associated with inhibition of cell proliferation, invasion and migration by inactivation of AKT and ERK [71].

Forkhead box P1 (FOXP1): Studies have found that rare mutations in this gene are associated with autism [72], and there is considerable evidence that FOXP1 protein expression is regulated by a PI3K/Akt/p70S6K signaling cascade [73].

SH3 and multiple ankyrin repeat domains 3 (SHANK3): Several studies have found rare single-gene variations in the SHANK3 gene in autism [74, 75]. However, Italian IMGSAC and Chinese Han studies claimed to find no genetic association or rare variations in the SHANK3 gene in autistic patients [76]. Pharmacological activation of Akt, and inhibition of CLK2, relieves synaptic deficits in SHANK3-deficient, as well as PMDS patient-derived, neurons. This suggests a strong relationship between SHANK3 and the Akt pathway [77].

Ubiquitin-protein ligase E3A (UBE3A) produces ubiquitin-protein ligase (E6AP) that is involved in targeting proteins for degradation within cells [78]. Mutations in the UBE3A gene are found in patients with Angelman syndrome (AS) [79]. AS is characterized by impaired motor and cognitive development, sleep disturbance, clumsy gait, seizures and irregular behaviors such as flapping and bursts of laughter. There is a phenotypic overlap between

autism and AS, and dysregulation of UBE3A may play a role in the causation of autism [80]. UBE3A functions as a co-activator to regulate PI3K-Akt signaling pathway [81].

NHE9 (SLC9A9) is an endosomal cation/proton antiporter with orthologues in yeast and bacteria. Rare, missense substitutions in NHE9 are genetically linked to autism, but have not been functionally evaluated. Loss-of-function mutations in NHE9 may contribute to autistic phenotype by modulating synaptic membrane protein expression and neurotransmitter clearance [82]. In addition, endosomal NHE9 is mechanistically involved in the pathophysiology of autism [83]. Specifically, in glioblastoma cells, altered NHE9 blocks proton leakage from endosomes, causing them to become more acidic. This changes the transportation dynamics of proteins in the cell [98]. Epidermal growth factor receptor (EGFR) signaling is a potent driver of glioblastoma, a malignant and lethal form of brain cancer. Data demonstrate that endolysosomal pH is critical for receptor sorting and turnover. By functioning as a leak pathway for protons, the Na+/H+ exchanger NHE9 limits luminal acidification to circumvent EGFR turnover and prolongs downstream signaling pathways [84].

MECP2 Rett syndrome (RTT), a neurodevelopmental disorder that commonly involves autism, is caused by mutations in this gene. The expressed protein, MECP2, is important for nerve cell maturation and both activates and represses transcription [85].

It should be recognized that the genes represented here are a small representation of the hundreds of de novo and familial risk genes, copy number variants, and epigenetic modifiers have been identified through linkage analysis, genome-wide association studies and exon and whole-genome sequencing of individuals with autism over the past few years [86–89].

3.3. Biomarkers associated with autism

3.3.1. Growth factors

3.3.1.1. EGF

An increased frequency of EGF single-nucleotide polymorphisms has been reported in children with autism [90]. Lower plasma EGF levels have been found in adults with autism [91], as well as in children [92, 93]. But these data appear to be uncertain, as one report of younger children with autism showed increased levels of EGF [94]. Our lab found reduced levels of EGF in an autistic group, and these decreased levels were associated with the inflammatory marker HMGB1 and severity of hyperactivity in individuals with autism [94].

3.3.1.2. HGF

Decreased serum levels of HGF were found in male adults with high-functioning autism [95]. Our lab found HGF decreased in the serum of older autistic children with co-occurring GI disease when compared to non-autistic children with GI disease, autistic children without GI disease and healthy controls (non-autistic children without GI disease) [96]. The c-Met promoter variant rs1858830 reflects a common single-nucleotide polymorphism that increases the risk of ASD and is distinctively associated with ASD in individuals with co-occurring GI dysfunction [97]. These data strongly suggest that decreased MET function is due to the

ASD-associated common genetic variant rs1858830, which predisposes a subset of ASD individuals that exhibit GI problems. These results may be one reason for the association between gastrointestinal issues and autism [98].

3.3.2. Receptors

3.3.2.1. EGFR

Our lab measured soluble EGFR in individuals with autism. We found that EGFR levels were significantly higher in the autism group. These receptor levels also correlated significantly with the inflammatory marker HMGB1 and several autism behavioral symptoms, including hyperactivity [99]. We found that EGF levels (described above) also correlated significantly with HMGB1 and hyperactivity severity. This helps to confirm a possible etiological relationship between the receptor (EGFR) and its ligand (EGF). In glioblastoma cells, when researchers administered drugs that countered NHE9 proteins (above), as well as drugs that countered EGFR proteins, the cells were more readily killed, compared to cells that were only treated with EGFR-countering medication [84]. This suggests a relationship between NHE9 and EGFR, and, also, if EGFR is elevated, in autism, and this elevation is associated with the etiology of autism, it might represent an important way to lower EGFR levels.

3.3.2.2. cMET

c-MET/HGF signaling is directly related to HGF expression via a positive feedback mechanism A polymorphism in the upstream region of the hepatocyte growth factor receptor, c-Met, identified in a study involving 1231 autistic children plus appropriate controls [100], shows a deficiency in c-Met protein level which corresponds to reduced transcription rate, as well as a downregulation of HGF production [101, 102]. Also, MET receptor protein expression is decreased in the postmortem brain of individuals with the MET 'C' allele [103], which further substantiates its association with the etiology of autism.

3.3.3. MAPK and Akt pathway proteins

3.3.3.1. MAPK proteins

Recent advances in autism genetics and progress in the study of animal models have provided evidence suggesting that Akt and MAPK pathway proteins are adversely affected in individuals with autism [104]. Genome-wide association studies [105] and genomic copy number variant (CNV) analyses have identified enrichment in gene sets involved in RAS (rat sarcoma)/MAPK signaling and kinase activation in ASD individuals [106]. It has been suggested in an animal model that activation of this pathway in peripheral lymphocytes may serve as a marker for central nervous system (CNS) ERK activity, and possibly autistic behavior [107], and there is evidence of developmental abnormalities in neurogenesis and behavioral impairment associated with the 16p11.2 chromosomal deletion, which is associated with ERK dysregulation [108]. Therefore, disruption of the ERK (MAPK) signaling pathway may be an important mediator of abnormal social behavior in individuals with autism.

In a mouse model, researchers found a significant association between juvenile social behavior and phosphorylated mitogen-activated protein kinase (p-Mek) and p-Erk levels in the prefrontal cortex, but not in the cerebellum [105]. Others found that neuronal cell differentiation and maturation, and unchanged apoptosis, are associated with the presence of amplified Mapk/Erk, although these data have since been retracted [109]. However, other studies have demonstrated that decreased Mapk/Erk activation can also disrupt stem cell proliferation [110–112] and may be associated with autistic behavior. This conflict suggests that dysregulation of the MAPK/ERK pathway may be associated with separate autism subpopulations, such that some populations have over-activation of MAPK, and others are characterized by lower activation.

The Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Database lists an array of highly associative genes associated with the MAPK/ERK pathway that are likely major contributors to ASD pathophysiology [113].

3.3.4. Akt proteins

3.3.4.1. Akt

Akt and mTOR, as well as related pathway proteins, have been found to be decreased in autistic groups [114]. Full-length PI3K, Akt and phosphorylated and total mTOR were reduced in the fusiform gyrus of a small group of autistic individuals [115]. Our lab has found significantly decreased levels of phosphorylated Akt in individuals with autism, and these low Akt levels correlated significantly with high EGFR and low GABA [116]. The low Akt levels in this study also correlated with high symptom severity. Our results suggest that the Akt pathway, starting with aberrant EGFR signaling, and followed by dysfunctional downstream Akt, may be involved in the etiology of autism.

3.3.4.2. PTEN

The gene for PTEN, the phosphatase that suppresses P13K/AKT signaling, is mutated in a small percentage of autistic individuals. In one study, all PTEN mutations from cases of PHTS (hamartoma tumor syndrome) appeared to disrupt the ability to inhibit AKT signaling, at the same time that PTEN expression was significantly reduced [117]. Inherited dominant PTEN mutations have been identified in patients with Cowden syndrome and Bannayan-Riley-Ruvalcaba syndrome, conditions that are often grouped together as PTEN hamartoma tumor syndrome (PHTS) [118], and inhibition of mTORC1 with the inhibitor rapamycin suppresses anatomical and behavioral abnormalities in PTEN knockout mice, suggesting a strong relationship between mTOR and PTEN [119], as well as an association with the etiology of autism.

3.3.4.3. MECP2

This protein, which is deficient in RETTs, may regulate PTEN expression with the help of a specific miRNA, miR-137. Conversely, PTEN can indirectly regulate MECP2 via the transcription factor, CREB and miR-132 [120].

3.3.4.4. mTOR

Significant decreases in protein expression and phosphorylation of mTOR, as well as components of its downstream signaling pathways, have been found in autism [121]. When mTOR is disrupted or decreased, downstream, effectors of protein translation are also reduced [122]. Mutations in TSC1, TSC2, NF1 and PTEN lead to an over-activated PI3K-mTOR pathway, as well as autism-relevant behaviors, tuberous sclerosis, neurofibromatosis and/or macrocephaly [123]. PI3K-mTOR signaling is also over-activated at synapses of fragile X syndrome (FXS) mice [124] and in humans with FXS [125]. mTOR signaling and protein synthesis are also impaired in a mouse model of Rett syndrome [126, 127]. Our lab found significantly high mTOR levels in individuals with autism, and these high levels correlated significantly with decreased oxytocin [128]. These data, collectively, suggest that mTOR may serve as a good marker for subgroups of individuals with autism.

3.3.4.5. TSC1 and TSC2

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder caused by mutations in either the TSC1 or TSC2 gene [122, 129]. Tsc1+/− and Tsc2+/− mice, both male and female, exhibit deficient social interaction, which is reversed by pharmacological inhibition of mTOR with rapamycin [130], demonstrating the strong relationship between TSC and mTOR.

4. Potential therapy for autism associated with Akt and MAPK pathway alteration of genes and/or proteins

4.1. Akt pathway

As outlined above, mutations in PTEN, NF1 or in the TSC 1 and 2, cause an over-activation of the PI3K/Akt/mTOR pathway, leading to autism-related behavior. Furthermore, fragile X syndrome (FXS), a common inherited form of mental retardation and the leading cause of autism, has been associated with over-activation of the PI3K-mTOR pathway. On the other hand, low levels of PI3K/Akt/mTOR activity are linked with Rett syndrome (RTT), a rare form of autism-associated disease. These data suggest that therapy designed to alter these pathway genes and/or proteins may be affective at altering autistic behaviors.

On the basis of observations that mTOR signaling, synaptic plasticity and protein translation are over-activated in these disorders, compounds that can potentially inhibit the mTOR pathway represent promising therapeutic candidates.

However, under-activation of this pathway has been reported in RTT. So, when developing therapy for RETTs, it may be important to use therapy that will raise mTOR levels.

Because mTOR has been found to be both high and low, depending on the syndrome, it is possible that altered mTOR levels may be associated with certain ASD behaviors and/or subpopulations, and therefore may provide a means for subgrouping those in the autistic spectrum.

For mTOR over-activation, the mTORC1 inhibitor rapamycin has shown promising results in PTEN KO mice [119] and TSC2 mice [131]. Other inhibitors of mTOR have been tried.

For instance, treatment of Fmr1 (protein of fragile X syndrome) knockout mice with temsirolimus, an mTOR inhibitor, prevented object recognition memory deficits and reduced audiogenic seizure susceptibility in mice [132].

Elevated phosphorylation of translational control molecules and exaggerated protein synthesis in fragile X mice were corrected through the targeting of S6K1 (ribosomal protein S6 kinase beta-1 (S6K1), also known as p70S6 kinase (p70S6K)), a serine/threonine kinase that acts downstream of PIP3 and targets the S6 ribosomal protein. Deletion of S6K1 prevents a broad range of fragile X phenotypes, including exaggerated translation and abnormal dendritic spine morphology [133]. Also, targeting downstream mTOR signaling, such as eukaryotic translation initiation factor 4E (eIF4E), reversed autism [122]. Thus, interventions that target mTOR signaling should be at the leading edge of future translational research for autism.

GSK3 (isoforms GSK3α and GSK3β) is a serine/threonine kinase that is controlled by inhibitory serine phosphorylation induced by both Akt and MAPK pathways [134]. In Fmr1 knockout mice, GSK3 phosphorylation was reduced in several brain regions, resulting in elevated GSK3 signaling [135]. Lithium is a GSK3 inhibitor that both increases the inhibitory serine phosphorylation of GSK3 and directly inhibits GSK3 activity [136, 137]. Particularly with chronic administration, in Fmr1 knockout mice, lithium was found to be able to correct abnormal behavior, such as hyperactivity [138]. Also, specific GSK3 inhibitors SB216763 141, TDZD-8 and VP0.7 corrected hippocampus-dependent learning deficits in fragile X mice [139, 140].

RTT is characterized by loss of function of the X-linked MECP2 gene [141]. Multiple studies, using different knockout mouse models, indicate that the pathological deficit in RTT is associated with the structure and function of synapses, synaptic transmission and plasticity [142, 143]. BDNF, the gene encoding the BDNF protein, is one of the first recognized direct targets of MECP2 transcriptional regulation [144]. BDNF, through the receptor tropomyosin-related kinase B (TrkB), activates intracellular signaling cascades, Akt and MAPK, critical for neuronal development, synaptic maturation, and learning and memory [145]. Therefore, one direction of potential therapy for MECP2 deficiency is the trial and use of drugs that boost BDNF. Compounds that boost BDNF levels such as fingolimod and glatiramer acetate (copaxone), both FDA-approved drugs for the treatment of multiple sclerosis, lead to an increase in BDNF expression and activation of TrkB/Akt downstream signaling pathways [146].

Insulin growth factor-1 (IGF-1) binds to the IGF-1 receptor and also activates intracellular signaling cascades, similar to those triggered by BDNF activation of TrkB receptors, by modulating synaptic plasticity and neuronal maturation through PI3K-Akt and MAPK pathways [147]. Unlike BDNF, IGF-1 permeability through the blood-brain barrier makes it an attractive compound for therapy. Based on these promising leads, a phase 2 double-blind placebo-controlled clinical trial is underway to treat 3- to 10–year-old RTT patients with full-length IGF-1 [148].

4.2. MAPK pathway

ERK levels, for the most part, are decreased in autism. Different classes of antidepressants rapidly increase ERK [149] and CREB [150] activity. This supports the involvement of the MAPK pathway in autism and may suggest the need to develop alternative therapies that increase ERK levels.

Supplemental zinc, which has been found to increase ERK levels, has an antidepressant-like effect. This zinc activity may be dependent on the activation of ERK and PI3K/GSK3β pathways [151].

4.3. Neurotransmitters

Multiple studies have documented low levels of neurotransmitters (dopamine, 5-HT, noradrenaline) in autopsy RTT brains and in MECP2-deficient mice [152]. Desipramine, an antidepressant that blocks the uptake of noradrenaline and has been shown to reverse the depletion of tyrosine hydroxylase in the brainstem, helps the regulation of breathing and extending the life span of male MECP2 knockout mice [153].

The NMDA-type of glutamate receptor is altered in MECP2 knockout mice [154], and the FDA-approved NMDA receptor antagonist ketamine has been useful in improving RTT-like phenotypes in MECP22 knockout mice [155].

Studies have shown that GABAergic signaling is also impaired in MECP2-deficient mice [156], and selective deletion of MECP2 in GABAergic neurons has led to impaired GABAergic transmission, cortical hyperexcitability and several neurological features of RTT and autism [157]. Vigabatrin is an antiepileptic drug that irreversibly inhibits GABA transaminase, inhibits GABA catabolism and thereby increases GABA levels. The drug is already FDA approved for use in epilepsy syndromes. Our lab has shown that decreased Akt levels are associated with low GABA [116], suggesting that therapy designed to increase Akt levels may also increase GABA.

4.4. Oxytocin

The neuropeptide oxytocin (OXT), a natural brain peptide produced in the hypothalamus, has been implicated in the pathophysiology and possibly the etiology of autism [158]. There is a significant association of a sequence variant in the OXT receptor gene with Asperger's syndrome [159]. However, the molecular mechanisms underlying the role of OXT in autism remain unclear. Recent studies have shown that OXT plays an important role in reducing behavioral deficits in an ASD-like mouse model, mediated by inhibiting the ERK signaling pathway and its downstream proteins [160]. OXT increases the salience of social stimuli, promotes parental nurturing and social bonds and, therefore, has received considerable attention as a potential treatment for social deficits in autism. Recent studies in mice have demonstrated that a mutation in Cntnap2 (the gene that encodes contactin-associated protein-like 2), which, in humans, may result in autism, displays robust social deficits and reduced brain OXT. In this model, daily intranasal OXT treatment improved later social engagement [161].

Acute intranasal OXT may temporarily enhance social cognition, empathy and reciprocity in individuals with autism [160]. In a recent small double-blind study, those taking OXT spray therapy had significantly reduced autism core symptoms specific to social reciprocity [162]. However, other recent clinical trials have yielded mixed results [161, 163].

Recent studies suggest that, in addition to its role in the brain, OXT may also have an important neuromodulatory role in the gut by activating the PI3K/Akt pathway in a dose- and time-dependent manner [164]. Our lab has found decreased plasma oxytocin in individuals

with autism, and these oxytocin levels correlated with high mTOR, suggesting a possible relationship between oxytocin and aberrant Akt pathway signaling [128].

In summary, because of the diversity of autism symptoms and behaviors, it is likely that drugs, based on altering genes or proteins of the Akt and/or MAPK pathways, will be most effective after biomarker screening. Marker screening may also provide an opportunity to identify and study subgroups of individuals with autism.

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