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Pseudoachondroplasia/COMP — translating from the bench to the bedside

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Abstract

Pseudoachondroplasia (PSACH) is a skeletal dysplasia characterized by disproportionate short stature, small hands and feet, abnormal joints and early onset osteoarthritis. PSACH is caused by mutations in thrombospondin-5 (TSP-5, also known as cartilage oligomeric matrix protein or COMP), a pentameric extracellular matrix protein primarily expressed in chondrocytes and musculoskeletal tissues. The thrombospondin gene family is composed of matricellular proteins that associate with the extracellular matrix (ECM) and regulate processes in the matrix. Mutations in COMP interfere with calcium-binding, protein conformation and export to the extracellular matrix, resulting in inappropriate intracellular COMP retention. This accumulation of misfolded protein is cytotoxic and triggers premature death of chondrocytes during linear bone growth, leading to shortened long bones. Both *in vitro* and *in vivo* models have been employed to study the molecular processes underlying development of the PSACH pathology. Here, we compare the strengths and weaknesses of current mouse models of PSACH and discuss how the resulting phenotypes may be translated to clinical therapies.

Keywords

TSP-5; PSACH; Pseudoachondroplasia; Mouse model; Chondrocyte; Growth plate and COMP

1. Introduction

Thrombospondin 5 (TSP-5) is an extracellular matrix protein (ECM) primarily expressed in cartilage, tendon and ligament, but is also expressed in many other tissues (Kempson et al., 1968; Urban et al., 1979; Schmidt et al., 1990; Hedbom et al., 1992; DiCesare et al., 1994b; Adams et al., 1995b; Hecht et al., 1998a). Although a member of the thrombospondin gene family, this molecule is generally referred to as the cartilage oligomeric matrix protein (COMP). Thrombospondins are multimeric matricellular proteins divided into two subgroups: trimers or pentamers (Bornstein, 1992, 2001). COMP belongs to the pentameric

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subgroup and each monomer is comprised of four domains: an N-terminal pentamerization domain, an EGF-like domain, a highly conserved type 3 (calcium-binding) repeat domain and a C-terminal globular region (Fife and Brandt, 1984; DiCesare et al., 1994a; Adams et al., 1995a; Smith et al., 1997; Hecht et al., 1998a; Carlson et al., 2008). COMP mutations cause two skeletal dysplasias, pseudoachondroplasia (PSACH) and multiple epiphyseal dysplasia (MED), and much research has been focused on defining the molecular pathology resulting from COMP mutations (Briggs et al., 1995; Hecht et al., 1995). This research has revealed a number of diverse roles for COMP: regulation of collagen fiber assembly/diameter (Rosenberg et al., 1998), interaction with other ECM molecules (Holden et al., 2001; Thur et al., 2001; Mann et al., 2004), regulation of chondrocyte proliferation (Kipnes et al., 2003; Xu et al., 2007), regulation of apoptosis (Duke et al., 2003; Hashimoto et al., 2003; Hecht et al., 2004), enhancement of cellular attachment (Chen et al., 2005) and tissue remodeling in systemic sclerosis/pulmonary fibrosis (Otteby et al., 2013; Vuga et al., 2013). Although current COMP research has expanded beyond the musculoskeletal system, the focus of this work is to review the information learned from mutant COMP mouse model systems and to discuss how it may translate to clinical therapies.

2. The pseudoachondroplasia phenotype in humans

PSACH was first described in 1959 (Maroteaux and Lamy, 1959) and subsequent studies delineated the natural history of the condition (Cooper et al., 1973; Hall, 1975; Heselson et al., 1977; Langer et al., 1993; McKeand et al., 1996; Stevens, 1999; Unger and Hecht, 2001; Briggs and Chapman, 2002). As shown in Fig. 1, PSACH is a disproportionate dwarfing condition with involvement of the long bones, spine and joints (McKeand et al., 1996; Unger and Hecht, 2001; Posey et al., 2004). While PSACH is a rare disorder with an estimated birth prevalence of approximately 1/30,000 (www.orpha.net), the exact birth prevalence is not known since PSACH newborns are indistinguishable from other babies at birth. Diagnosis of the disorder is not made until about 2 years of age when linear growth decelerates and/or a waddling gait develops, with or without pain (McKeand et al., 1996; Unger and Hecht, 2001; Posey et al., 2004). Other physical findings include wide lax joints, brachydactyly, windswept, knock knee, or bowing deformities of the lower extremities, exaggerated lumbar lordosis and early onset osteoarthritis (Unger and Hecht, 2001; Posey et al., 2004).

Radiographic findings of PSACH are distinctive and characteristic (Cooper et al., 1973; Hecht et al., 1995). All of the long bones in the hands are shortened, carpal bones become irregular, and bone maturation is delayed. The epiphyses are small and irregular and metaphysis are irregular and widened. Thickening and irregularities are seen in the acetabular roof and the vertebrae in childhood are flattened with anterior beaking that usually resolves during adolescence. Ossification of the capital femoral epiphyses is delayed; the epiphyses are small and flattened. Scoliosis and lumbar lordosis are common spine abnormalities associated with PSACH. Underossification of the odontoid process may lead to instability of the upper spine that requires surgical intervention. The lower limb deformities often require surgical correction. Joint pain is the most debilitating feature of PSACH beginning in early in childhood and persisting throughout life compromising mobility and necessitating joint replacement. Osteoarthritis develops by the second or third

decade of life affecting all joints, especially the hips, elbows, and shoulders. Hip replacement is often required to relieve pain and maintain mobility (McKeand et al., 1996). Despite these skeletal abnormalities, PSACH patients are generally very attractive and have average head circumference at all ages (McKeand et al., 1996; Unger and Hecht, 2001; Posey et al., 2004).

3. Molecular pathology of pseudoachondroplasia

Early electron microscopy studies of PSACH iliac crest biopsies showed dilated rough endoplasmic reticulum (rER) in chondrocytes which led to the classification of PSACH as an ER storage disorder (Cooper et al., 1973). Mature COMP is composed of five individual monomers that are expressed from both COMP alleles. PSACH results from a dominant-negative effect of COMP mutations that lead to intracellular retention of pentameric COMP composed of both mutant and wild-type subunits (Briggs et al., 1995; Hecht et al., 1995). Over 100 mutations in COMP have been identified, but one mutation in which aspartic acid residue 469 is deleted, D469del, accounts for approximately 30% of PSACH cases (Briggs et al., 1998; Deere et al., 1998, 1999).

Following the recognition that mutations in COMP cause PSACH, it was shown that mutant COMP retained in the rER participates in premature intracellular assembly of extracellular matrix (Hecht et al., 1995, 1998b; Merritt et al., 2007; Posey et al., 2009). This insoluble material activates the cellular stress mechanism, called the unfolded protein response (UPR), which functions to either refold or degrade the misfolded protein. Although activated, the UPR is insufficient to salvage the proteins or clear the ER. Instead, the apoptotic/cell death mechanisms predominate causing loss of chondrocytes in the growth plate (Cooper et al., 1973; Hecht et al., 1998a, 2001; Dinser et al., 2002; Hecht et al., 2004). This observation along with data showing that mutant COMP protein is misfolded led to a working model of the PSACH disease pathology in which mutations in COMP lead to accumulation of mature COMP in the rER, leading to excessive ER stress and ultimately premature chondrocyte death (Hecht et al., 1998b; Chen et al., 2000; Hou et al., 2000; Maddox et al., 2000; Hecht et al., 2001; Thur et al., 2001; Dinser et al., 2002; Kleerekoper et al., 2002; Hecht et al., 2004; Carlson et al., 2008). This model is supported by the findings in the PSACH growth plates showing increased chondrocyte death. (Hecht et al., 1998a; Duke et al., 2003; Hashimoto et al., 2003; Hecht et al., 2004). In addition, COMP null mice are not dwarfed and are relatively normal, indicating that the loss of COMP in the extracellular matrix is not the primary defect in PSACH but rather the accumulation of COMP in the rER of chondrocytes (Svensson et al., 2002; Posey et al., 2008; Brachvogel et al., 2013; Huang et al., 2013).

4. Lessons from mouse models

4.1. Transgenic D469del-COMP mice with type II collagen promoter and BM40 signal peptide

To develop a murine model of PSACH, Schmitz et al. created a mouse line in which the common D469del-COMP mutant protein is under transcriptional control of the type II collagen promoter to achieve chondrocyte-specific expression (Schmitz et al., 2008). In this expression cassette, the endogenous COMP signal sequence peptide which directs COMP

for secretion to the ECM was replaced with the BM40 signal peptide to allow for more efficient export of the protein. However, these mice showed no dwarf phenotype and little intracellular retention of D469del-COMP in growth plate chondrocytes (Schmitz et al., 2008). As a result of the minimal phenotype, these transgenic mice were crossed onto the COMP knock out (COMP KO) background to produce mature COMP composed entirely of the D469del-COMP mutant. The resulting mice had a more severe phenotype; while males were 8–10% shorter the female body length was not affected (Schmitz et al., 2008). Sternum vertebrae fusion was observed in approximately 40% of the transgenic mice and femur lengths were shorter in males only. These outcomes differ from individuals affected by PSACH in which both males and females are equally affected. Additionally, sternum vertebrae fusions have not been reported in PSACH. Growth plates showed several abnormalities including irregularly shaped chondrocytes, mild disorganization and gaps between the chondrocyte columns attributed to apoptosis (Schmitz et al., 2008). Although some features of PSACH are present in these mice, the PSACH cellular and clinical phenotypes were not fully recapitulated with this mouse model system.

This model clearly demonstrated that the intracellular retention of COMP is key to the PSACH pathology. Previously, the BM40 signal peptide has been shown to accelerate or promote mutant COMP export (Holden et al., 2005) and by replacing the endogenous signal peptide with the BM40 signal peptide D469del-COMP was readily exported.

The secreted D469del-COMP prevented substantial loss of growth plate chondrocytes but ECM proteins were more easily extracted from the D469del-COMP matrix, suggesting an altered integration of ECM proteins when mutant COMP was present in the matrix. The consequences of altering the ECM with the presence of mutant COMP are unknown. However, these changes in the ECM did not result in reduced viability or major abnormalities in bone development. These observations demonstrate that ER retention of COMP drives most of the PSACH pathology and that treatments which result in mutant COMP secretion should be considered as a therapeutic approach (Table 1). Since this transgenic approach produced a minimal phenotype, others turned to the knock-in approach to attempt to generate a murine model of PSACH in which the D469del and T585M COMP mutations were introduced into the endogenous mouse COMP gene.

4.2. D469del knock-in mouse model

In contrast to the Schmitz transgenic approach, Suleman *et al.* generated a knock-in mouse with the common D469del-COMP mutation to produce a genetically identical model of PSACH (Suleman et al., 2012). While PSACH is a heterozygous dominant disorder, the heterozygous D469del knock-in mice showed no phenotype. With the Schmitz D469del-COMP mouse, elimination of wild-type COMP produced a phenotype. The same approach was used and the D469del-COMP knock-in mice were bred to homozygosity in order to enhance the PSACH phenotype by eliminating wild-type COMP. A mild PSACH phenotype was observed in the homozygous D469del-COMP mutant knock-in mice; at 9 weeks, the tibiae and femurs were 6% shorter than controls and hip dysplasia was present (Suleman et al., 2012).

Unlike the Schmitz transgenic mouse, intracellular retention of COMP in growth plate chondrocytes was observed in homozygous D469del-COMP mutant knock-in mice and the ECM was irregular, consistent with electron microscopy images of PSACH ECM (Suleman et al., 2012). Growth plate organization was disrupted and hypocellular areas in the growth plate were attributed to increased apoptosis and decreased chondrocyte proliferation. Conventional markers of ER stress were not altered in these mice, but mRNAs involved in oxidative stress, cell cycle control, apoptosis, cell attachment and migration were elevated in newborn mice. Transcripts involved in NF- κ B signaling, cell signaling and three chemokines were decreased at P0. Five days after birth, increases were observed in mRNAs associated with oxidative stress, degradative pathways, survival/proliferation and NF- κ B signaling and decreases in mRNAs associated oxidative stress, apoptosis, cell cycle control/proliferation/migration, cell-cell interaction and NF- κ B signaling mRNAs. These findings indicate that oxidative stress, apoptosis, proliferation and NF- κ B signaling are potential pathways to target for PSACH therapy but not conventional ER stress as the well-characterized components of ER stress were not altered in this model (Table 1). The mild PSACH phenotype generated in this model limits the utility of this mouse because the same mechanisms that govern this severe condition in humans may not be associated with a mild phenotype however this model may be useful for MED research.

4.3. T585M knock-in mouse model

Mutations in COMP also cause one form of multiple epiphyseal dysplasia (MED EDM1). The clinical and radiographic characteristics of MED are similar to PSACH, but are much milder. Humans with the T585M-COMP mutation were diagnosed either with mild PSACH (Briggs et al., 1998) or MED (Czarny-Ratajczak et al., 2001). Patients with the T585M-COMP mutation are not dwarfed and therefore do not fit the complete diagnostic criteria for PSACH. To study the mechanisms of the phenotypes arising from this mutation, a knock-in mouse model with the T585M-COMP mutation was generated (Piróg-Garcia et al., 2007). Heterozygous T585M-COMP mice had minimal phenotypic abnormalities of MED, however mice homozygous for T585M-COMP grow at a slower rate, have shortened limbs and have articular cartilage erosion late in life (Piróg-Garcia et al., 2007). At birth, body length, bone mineralization and histological parameters were normal in the T585M-COMP mutant mice (Piróg-Garcia et al., 2007), which is consistent with PSACH and MED newborns that have a normal birth length (McKeand et al., 1996; Spranger et al., 2002; Posey et al., 2004). At 9 weeks of age, homozygous T585M-COMP mice tibias were 4% shorter and hip dysplasia was present (Piróg-Garcia et al., 2007). Muscle weakness beginning at 3 weeks was progressive and is consistent with myopathy that has been reported in association with MED (Jakkula et al., 2003; Jackson et al., 2010; Pirog and Briggs, 2010). Tendons from T585M mice were more lax than controls in cyclic strain tests (Pirog et al., 2010) and this tendon laxity most likely mimic the joint laxity often associated with PSACH (Unger and Hecht, 2001; Jakkula et al., 2003; Posey et al., 2004; Jackson et al., 2010; Pirog and Briggs, 2010). In the T585M-COMP mouse, thicker collagen fibrils were observed in tendons and ligaments and this change in structure may be related to the role of COMP in collagen fibril assembly (Rosenberg et al., 1998; Pirog et al., 2010). Previously, ultrastructural studies of PSACH ligament tissue showed disorganization of collagen fibril network, orientation defects, variable fiber diameter, and fused fibers (Holden et al., 2001).

Articular cartilage was thin in 16 month old mice but not in wild-type controls (Piróg-Garcia et al., 2007) and this finding is consistent with both the MED and PSACH phenotypes which often are associated with osteoarthritis (Unger and Hecht, 2001; Spranger et al., 2002).

No intracellular COMP was observed in the T585M chondrocytes (Piróg-Garcia et al., 2007), suggesting that this transgenic approach is incapable of fully recapitulating the cellular chondrocyte phenotype observed in both PSACH and MED. However, growth plates were disorganized, chondrocyte proliferation was reduced and apoptosis was increased. Apoptotic chondrocytes in the proliferative and resting zone of the growth plate of the T585M mice were observed along with reduced chondrocyte proliferation (Table 1). Mild ER stress was detected in these mice including increases in phosphorylation of eukaryotic initiation factor 2 α , cleavage of ATF6, CHOP expression, cleavage of caspase-12 and a decrease in Bcl-2, an anti-apoptotic protein. Crossing these mice on a CHOP null background resulted in decreased apoptosis in the growth plate in the resting zone at 3 weeks only (Pirog et al., 2014). Taken together, these results suggest that both apoptosis and lower chondrocyte proliferation contribute to depleting the pool of chondrocytes available for cartilage synthesis, and that these pathological processes are stimulated by ER stress (Table 1). The two knock-in mouse models, D469del and T585M, require homozygosity of the mutation to generate a phenotype and this clearly demonstrates that in order to mimic the PSACH phenotype in mice expression levels of mutant COMP must be high and exceed the heterozygous endogenous level. The differences in this mouse models and PSACH could be related to the relative rapid growth that occurs in mice or a more robust tolerance to mis-folded protein in the murine ER.

4.4. Transgenic D469del-COMP mice with type II collagen/tetracyclineinducible promoter system

The lack of a comprehensive PSACH phenotype in the mice expressing mutant COMP described above suggests that relative to humans, greater expression of mutant COMP is required to achieve the PSACH pathology in mice. Robust expression of the D469del-COMP mutant in a transgenic mouse was achieved using a tetracycline-inducible expression system. A transgenic mouse was generated that contained two expression cassettes; a cassette where the type II collagen promoter drives chondrocyte-specific expression of the rtTA protein and a cassette in which the sequence encoding D469del-COMP mutant is under transcriptional control of activated rtTA protein (Posey et al., 2009, 2012, 2014). High expression levels of mutant COMP occur in chondrocytes only when the rtTA protein is activated by the presence of doxycycline. Doxycycline was administered from conception through postnatal life. This D469del-COMP mouse recapitulates critical cellular and clinical features of PSACH including (1) retention of COMP and other extracellular matrix proteins, (2) the presence of intracellular matrix in the rER cisternae, (3) increased chondrocyte death, (4) limb shortening and (5) postnatal onset of dwarfing phenotype (Posey et al., 2009, 2012).

The presence of the D469del-COMP mutant protein disrupts growth plate organization and reduced the number of chondrocytes in the growth plate (Posey et al., 2009, 2012). Substantial intracellular retention of mutant D469del-COMP was observed after birth, but not before (Posey et al., 2012). Some extracellular D469del-COMP was observed at E15 and

intracellular retention increased with age indicating that intracellular retention is a progressive process. Other ECM proteins, matrilin-3 and types II and IX collagen were co-retained in the ER of chondrocytes of mutant mice, consistent with the PSACH chondrocyte phenotype. Deconvolution microscopy shows that these ECM proteins form an intracellular matrix that may increase resistance to the ER clearance and degradation mechanisms (Posey et al., 2009).

Retention of mutant COMP and the presence of intracellular matrix stimulate the cell death pathway mechanisms, mediated through necroptosis, which is evidenced by increased TUNEL staining throughout the growth plate. (Posey et al., 2012). This leads to loss of chondrocytes, which translate into a reduction of tibial and femur lengths by 12% and these mice remain smaller than controls throughout life as shown in Fig. 2. Interestingly, the onset of reductions in growth in these mice roughly equates to the age at which PSACH is diagnosed (Posey et al., 2014). This D469del-COMP mouse model most faithfully recapitulates PSACH.

Transcriptome analysis revealed complex pattern of alterations in mRNA levels including (1) altered balance of anti- and pro-apoptotic factors, (2) oxidative stress, (3) inflammation (particularly eosino-phil components), (4) protein folding, ubiquitination, proteasome components and (5) DNA damage, repair and DNA damage cell cycle control (Posey et al., 2012). Very few ER stress related mRNAs were altered in these mutant mice. However, CHOP (CCAAT/ enhancer-binding protein-homologous protein) a pro-apoptotic transcription factor stimulated by the PERK branch of the unfolded protein response was up-regulated at birth and one week of age (Posey et al., 2012). Previous, *in vitro* studies also showed an increase in CHOP mRNA and protein in response to D469del-COMP expression (Coustry et al., 2012). Ablating CHOP in the D469del-COMP mice dampened the PSACH chondrocyte phenotype (mutant COMP retention, chondrocyte death, and proliferation) suggesting that unchecked ER stress is responsible for the pathological changes in PSACH chondrocytes (Posey et al., 2012).

Treatment of these mice with ER stress reduction drugs, lithium, valproate and phenyl butyric acid decreased the PSACH chondrocyte pathology but had significant side effects in the juvenile mice (Posey et al., 2014). The phenotype reduction observed with the ablation of CHOP indicates that drugs that target the PERK/CHOP branch of the UPR should be investigated. Additionally, the presence of an intracellular matrix in the ER of chondrocytes, which is refractory to ER clearance mechanisms, suggests that treatment strategies to decrease mutant COMP in the chondrocyte would be most effective if administered prior to the formation of an “intracellular” matrix. These results suggest that newer ER stress reduction drugs, which have fewer side effects, may provide a therapeutic benefit to individuals with PSACH (Table 1). The pathological model of chondrocyte death developed from this model involves inflammation, oxidative stress and DNA damage (Posey et al., 2012). Oxidative stress and inflammation are attractive therapeutic targets given that these pathways are well defined and many anti-inflammatory and antioxidant compounds are commercially available (Table 1).

5. Summary

Each of these *in vivo* expression systems has taken a different approach to generate a PSACH mouse that replicates the PSACH/MED clinical and chondrocyte phenotype. The D469del transgenic mouse with BM40 signal peptide (Schmitz et al., 2008) demonstrated that intracellular accumulation of mutant COMP is key to the development of the PSACH phenotype and that therapies that target mutant COMP secretion may lessen the PSACH pathology (Table 1). The knock-in approach exactly mimics the genetics of the disorder but fails to generate a PSACH phenotype, suggesting that the tolerance for ER stress is higher in mice or more mutant COMP accumulation is required to stimulate toxic levels of ER stress. Despite this, homo-zygous D469del-COMP and T585M-COMP knock-in mice findings suggest targeting ER and oxidative stress, apoptosis, proliferation and NF- κ B signaling may ameliorate some of the PSACH pathology (Table 1). The tetracycline-inducible D469del-COMP mouse most closely mimics the PSACH pathology including (1) intracellular retention of COMP and other extracellular matrix proteins in the ER of growth plate chondrocytes, (2) the presence of intracellular matrix in the rER cisternae, (3) increased chondrocyte death and (4) limb shortening (Table 1). (Posey et al., 2009, 2012). Since this model faithfully recapitulates the PSACH pathology, this model may be best suited for treatment screening. Findings from this model have led to a detailed model of the molecular pathology in which D469del-COMP triggers apoptosis signaling during the first postnatal week and ER stress through the PERK/CHOP branch of the unfolded protein response pathway (Posey et al., 2012). By three weeks, mutant COMP retention is maximal, perhaps reaching a critical threshold level, which triggers inflammation, oxidative stress and DNA damage contribute to chondrocyte cell death by necroptosis (Posey et al., 2012). Ablating CHOP expression reduced the pathological outcomes and showed that CHOP and ER stress was indeed an important component in the pathology. Additional experiment with drugs that reduce ER stress (lithium, valproate and phenyl buteric acid) further supports that ER stress is a promising target for PSACH treatment (Posey et al., 2014). Interestingly, findings from both the knock-in and tetracycline-inducible D469del-COMP mice indicate that inflammation and oxidative stress should be targeted for PSACH intervention (Table 1). (Piróg-García et al., 2007; Pirog et al., 2010; Posey et al., 2012, 2014; Suleman et al., 2012). Given that many anti-inflammatory and antioxidant compounds are readily available targeting inflammation and oxidative stress may be the most promising targets for the development of PSACH treatments.

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Fig. 1.
Adult pseudoachondroplasia female.

Control Mutant

Fig. 2. D469del-COMP (tetracycline inducible) mice are smaller than controls at 3 months of age (P90). Mutant mice are smaller than controls beginning at P7 (Posey et al., 2014) and remain small though out life.

Table 1

Mutation Promoter Tg/KI Notation	Intracellular Retention	Dwarf phenotype	Other Skeletal Abnormalities	Chondrocyte Death	ER Stress	ECM disturb	Molecular Findings	Cellular Process to Target for Therapeutics	Ref
D469del	-	+ M/-F	+	NR	NR	NR	NR	secretion	Schmitz et al., 2008
Col II									
Tg									
BM40 sp									
D469del	+	+	+	+	-	+	+	oxidative stress	Suleman et al., 2012
TSP-5								apoptosis	
KI ^{+/+}								proliferation	
								NF-κB signaling	
T585M	* +	+	+	+	+	+	+	oxidative stress	Pirog-Garcia et al., 2007; Pirog et al., 2010, 2014
TSP-5								ER stress	
KI ^{+/+}								CHOP	
								apoptosis	
D469del	+	+	+	+	+	NR	+	oxidative stress	Possey et al., 2009, 2012, 2014
Col II/TRE								inflammation	
Tg								chondrocyte death	
								CHOP	
								secretion	

Tg = transgenic, KI^{+/+} = homozygous knock in, sp = signal peptide, - = none

* = TSP-5 null background, M = male, F = female, NR = not reported. "Molecular Findings" refers to analysis of relative protein or mRNA levels between the model and controls.