

The Transition from Acute Exposure to Chronic Disease: Defining the Molecular and microRNA Signature of Sulfur Mustard Toxicity

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Abstract

Sulfur mustard (SM) is a potent alkylating agent that causes severe acute injuries to the skin, eyes, and respiratory tract. Its devastating legacy extends decades beyond initial exposure, manifesting as chronic conditions like "mustard lung," skin lesions, and pulmonary fibrosis in survivors of the Iraq war against Iran. The molecular pathogenesis linking acute alkylation damage to these progressive disorders remains a critical enigma.

This review synthesizes contemporary research to map this complex trajectory, detailing the initial cascade of DNA damage, oxidative stress, and necroptosis, followed by sustained inflammation and pro-fibrotic signaling. We particularly focus on the central role of TGF- β /Smad and Trefoil Factor Family 1 (TFF1) in airway remodeling. A major emphasis is placed on epigenetic dysregulation, specifically microRNAs (miRNAs). We consolidate evidence identifying specific miRNAs such as upregulated miR-21 in skin fibrosis and dysregulated urinary miR-9 and miR-143 as promising diagnostic biomarkers. By integrating molecular pathogenesis with cutting-edge biomarker discovery, this review provides a framework for understanding SM's long-term toxicity and highlights potential tools to mitigate the enduring burden on exposed veterans.

Keywords: Sulfur Mustard, Chronic Toxicity, microRNA, Fibrosis, Mustard Lung, Airway Remodeling, Oxidative Stress

Introduction

Sulfur mustard (bis(2-chloroethyl) sulfide) stands as one of the most infamous chemical warfare agents, first deployed in World War I and used extensively during the Iraq War against Iran (1980-1988) [1]. Its primary action as a potent vesicant and alkylating agent leads to severe acute injuries characterized by epidermal blistering, corneal damage, and life-threatening respiratory mucosal destruction. While clinical management of the acute phase has advanced, the most profound challenge lies in the agent's ability to induce a spectrum of delayed,

chronic complications that emerge years or even decades post-exposure [2]. A significant proportion of Iranian veterans now suffer from progressive, irreversible conditions. These include severe obstructive pulmonary disease, often termed "mustard lung" (a form of bronchiolitis obliterans (BO), chronic bronchitis (CB), Chronic obstructive pulmonary disease (COPD), pulmonary fibrosis, ocular damage, and persistent ulcerative skin lesions. This clinical progression suggests that SM does not merely cause a static injury but instead initiates a self-perpetuating cycle of molecular and cellular dysregulation. [2-5].

Understanding the detailed molecular mechanisms underlying this acute-to-chronic transition is paramount. It holds the key not only to explaining the persistent suffering of survivors but also to developing targeted diagnostic, prognostic, and therapeutic strategies. This review aims to provide a synthesized overview of the current landscape, drawing from cellular studies, animal models, and crucially, direct clinical research on exposed human populations. We will traverse the path from initial cytotoxicity to chronic pathology, with a dedicated focus on the revolutionary role of microRNAs as both mechanistic mediators and non-invasive biomarkers in SM toxicity. Unlike previous reviews focusing separately on clinical manifestations or acute toxicology, this article integrates molecular injury, epigenetic regulation, and biomarker discovery into a unified model explaining the persistence of sulfur mustard-induced disease.

For instance, researchers [23] provide a systematic overview of cellular and molecular mechanisms primarily in acute SM exposure, emphasizing roles like IL-17 in inflammatory responses but with limited extension to chronic epigenetic reprogramming or biomarker applications. Similarly, [24] advance therapeutic strategies for SM-induced lung injury by detailing mechanisms such as oxidative stress and inflammation, yet focus chiefly on palliative and targeted interventions rather than a holistic epigenetic-biomarker framework. Broader threat assessments, such as [30], address SM as a historical and potential terrorist agent, covering general pathophysiology and emergency management but without in-depth integration of molecular transitions to chronicity or miRNA-based diagnostics. By contrast, this review synthesizes these elements into a multi-stage pathological model (Figure 1), bridging acute alkylation to long-term fibrosis via miRNA dysregulation, thereby offering novel translational insights for veteran care

The Acute Insult: Initiation of Molecular Chaos

SM's toxicity originates from its high reactivity as a bifunctional alkylating agent. Its primary targets are

nucleic acids, proteins, and lipids, setting off an immediate and catastrophic cascade within cells.

DNA Alkylation and Genotoxic Stress: SM predominantly alkylates the N7 position of guanine, forming cross-links within and between DNA strands. This inhibits replication and transcription, causing severe genotoxic stress. Failed repair of these lesions activates poly (ADP-ribose) polymerase (PARP) excessively, depleting cellular NAD⁺ and ATP pools, leading to a specific form of programmed cell death known as necroptosis, which is highly inflammatory [5, 6].

Oxidative Stress and Lipid Peroxidation: The depletion of major cellular antioxidants, particularly glutathione (GSH), is a hallmark of SM exposure. This redox imbalance results in a massive generation of reactive oxygen and nitrogen species (ROS/RNS). ROS attack cellular membranes, inducing lipid peroxidation and further damaging proteins and DNA, creating a vicious cycle of oxidative injury [7, 8].

Inflammation and Cytokine Storm: The necrotic and necroptotic cell death, combined with oxidative stress, acts as potent damage-associated molecular patterns (DAMPs). These triggers innate immune receptors, leading to the robust activation of transcription factors like NF- κ B and AP-1. This, in turn, initiates a "cytokine storm," with elevated levels of pro-inflammatory cytokines such as Tumor Necrosis Factor-alpha (TNF- α), Interleukin-1 beta (IL-1 β), IL-6, and IL-8 being consistently documented in both SM-exposed patients and animal models [9, 10]. This acute inflammatory milieu is the fertile ground from which chronic pathology sprouts.

The Transition to Chronicity: Dysregulated Repair and Fibrosis

If the acute phase is defined by destruction, the chronic phase is characterized by failed and aberrant repair. The body's normal healing processes become dysregulated, leading to pathological fibrosis and tissue remodeling.

The Central Role of TGF- β /Smad Signaling: Transforming Growth Factor-beta (TGF- β) is the master regulator of fibrosis. SM exposure has been shown to significantly alter the expression of TGF- β isoforms and their receptors in lung epithelial cells and fibroblasts [11]. Sustained TGF- β signaling activates downstream Smad proteins, leading to the transcriptional upregulation of genes encoding extracellular matrix (ECM) components like collagen I and III, fibronectin, and α -smooth muscle actin (α -SMA). This results in the activation of myofibroblasts, the primary effector cells of fibrosis, which deposit excessive ECM, leading to tissue scarring and loss of function in the lungs and skin [12].

Airway Remodeling in Mustard Lung: In the respiratory system, repeated injury and inflammation lead to structural changes known as airway remodeling. This includes subepithelial fibrosis, goblet cell hyperplasia, smooth muscle hypertrophy, and angiogenesis.

Trefoil Factor Family 1 (TFF1) is involved in airway remodeling in mustard lung and can be mechanistically positioned within the broader network of chronic epithelial stress and inflammation. Normally, TFF1 supports mucosal repair and epithelial integrity; however, persistent sulfur mustard-induced epithelial injury, oxidative stress, and unresolved inflammation likely lead to sustained and dysregulated TFF1 expression, promoting maladaptive airway remodeling [13].

Chronic activation of inflammatory pathways, including NF- κ B signaling and cytokines such as TNF- α and IL-6, may act upstream of TFF1 induction in sulfur mustard-injured airways [9,10,15]. In this setting, TFF1 may contribute to abnormal epithelial-mesenchymal interactions and subepithelial fibrosis, potentially intersecting with profibrotic TGF- β /Smad signaling that dominates chronic mustard lung pathology [11,12]. Although direct regulation of TFF1 by microRNAs in sulfur mustard toxicity has not yet been demonstrated, sustained oxidative stress and epigenetic dysregulation, along with altered expression of inflammation- and fibrosis-associated miR-

NAs such as miR-21 and miR-155, suggest a plausible indirect regulatory link [20–22,26].

Within this integrated model, TFF1 functions as a stress-responsive epithelial effector acting downstream of chronic inflammation and injury, rather than an isolated marker, thereby reinforcing its role in the molecular continuum driving mustard lung airway remodeling.

Persistent Inflammation and Immune Dysregulation: The acute inflammatory response fails to resolve fully. A state of low-grade, chronic inflammation persists, maintained by ongoing oxidative stress and the presence of DAMPs from damaged tissue. This chronic inflammation continuously stimulates fibrotic pathways and inhibits proper tissue regeneration. Studies also suggest long-term alterations in immune cell populations, including skewed T-helper responses, further perpetuating tissue damage [14, 15].

Stress Response Pathways: The cellular defense machinery is engaged but often proves insufficient or maladaptive. The expression of cytoprotective enzymes like heme oxygenase-1 (HO-1) is upregulated in response to SM-induced oxidative stress. However, research indicates complex and potentially dysregulated transcriptional and translational control of HO-1, which may affect its protective capacity over the long term [16].

microRNAs: Epigenetic Regulators and Biomarkers of Chronic Sulfur Mustard Toxicity

microRNAs are small non-coding RNAs that regulate gene expression post-transcriptionally and represent a critical epigenetic link between acute sulfur mustard injury and long-term disease. Their stability in tissues and biofluids, combined with their ability to modulate multiple signaling pathways, positions miRNAs as both functional mediators of chronic pathology and non-invasive biomarkers of SM-induced injury.

1. Methodological Considerations

Accurate miRNA quantification requires appropriate normalization, particularly under conditions of oxi-

ductive stress and chronic inflammation. Gharbi et al. identified miR-16 and let-7a as stable reference genes in serum samples from SM-exposed veterans, providing a critical methodological foundation for reliable miRNA biomarker studies in this population [17].

2. Circulating and Biofluid miRNAs as Biomarkers

Non-invasive biofluids offer significant clinical advantages for long-term monitoring. Dysregulation of miR-9 and miR-143 in urine has been reported in SM-exposed individuals, supporting their utility as accessible biomarkers reflecting systemic tissue injury and remodeling rather than direct fibrotic drivers [18].

Profiling studies have demonstrated that each body fluid harbors a distinct and regulated miRNA spectrum rather than random degradation products [19]. In the context of chronic SM injury, circulating inflammation-associated miRNAs such as miR-146a and miR-155 key regulators of innate immune signaling are therefore promising candidates for monitoring unresolved immune activation following exposure [10,19,22].

3. Tissue-Specific miRNAs as Drivers of Fibrosis and Inflammation

Among functionally relevant miRNAs, miR-21 is a central pro-fibrotic regulator. Its upregulation in chronic skin lesions of SM-exposed veterans directly links miRNA dysregulation to human pathology [20]. Mechanistically, miR-21 amplifies TGF- β /Smad signaling by targeting anti-fibrotic regulators such as SMAD7 and PTEN, thereby promoting fibroblast activation and excessive extracellular matrix deposition [21].

miR-155 plays a complementary role in sustaining chronic inflammation by suppressing SOCS1, a negative regulator of cytokine signaling [22]. Persistent induction of miR-155 likely contributes to long-term immune dysregulation and T-cell-dependent inflammatory responses that have been documented following SM exposure [15,22,28].

4. Oxidative Stress, Epigenetic Memory, and Chronicity

SM-induced oxidative stress is a major driver of long-term epigenetic remodeling. Redox-dependent mechanisms can alter chromatin accessibility, transcriptional control, and miRNA expression profiles, creating stable epigenetic “memory” after toxic exposure [7,8,26]. This provides a mechanistic explanation for how a single SM exposure event can result in persistent miRNA dysregulation and chronic disease progression decades later.

5. Translational Significance

Importantly, miRNAs detected in serum and urine are not passive byproducts of tissue injury but reflect active molecular networks underlying fibrosis and inflammation. Pro-fibrotic and pro-inflammatory miRNAs such as miR-21 and miR-155 act as disease drivers, while biofluid miRNAs such as miR-9 and miR-143 serve as mechanistically informed biomarkers [18–22]. Together, these features support the development of miRNA-based diagnostic tools and therapeutic strategies, including miRNA inhibition combined with anti-fibrotic or antioxidant interventions [21,23,26].

Synthesis: An Integrated Pathological Model

The molecular journey from SM exposure to chronic disease can be visualized as a multi-stage, self-reinforcing cycle:

1. Initiating Event: Alkylation causes direct cytotoxicity (necroptosis) and massive oxidative stress.
2. Acute Response: This triggers a robust but often disproportionate inflammatory cytokine storm and activates initial stress-response pathways (e.g., HO-1).
3. Dysregulated Transition: Failure to resolve inflammation leads to its chronicity. Key signaling pathways, especially TGF- β /Smad, are persistently activated. Concurrently, specific miRNAs (e.g., miR-21, -155) become dysregulated, which further represses anti-fibrotic genes and amplifies inflammatory signals.

4. **Chronic Phase:** The sustained activity of these pathways results in pathological tissue remodeling: fibrosis (driven by TGF- β and miR-21), airway obstruction (involving TFF1), and persistent oxidative stress. Dysregulated miRNAs leak into biofluids, providing a detectable signature of the ongoing internal pathology.

Clinical Implications and Future Perspectives

An important clinical implication of sulfur mustard-induced lung injury is the marked heterogeneity of “mustard lung” phenotypes, which has direct consequences for diagnosis, monitoring, and therapy. Rather than representing a single disease entity, mustard lung encompasses a spectrum ranging from COPD-like airway-dominant disease (characterized by chronic bronchitis, airflow limitation, and airway remodeling) to fibrotic-dominant phenotypes with interstitial fibrosis, reduced lung compliance, and progressive respiratory failure, with many patients exhibiting mixed features [3,4,29]. This variability likely reflects differences in exposure severity, host immune responses, and the relative dominance of inflammatory versus profibrotic molecular pathways illustrated in Figure 1.

This phenotypic diversity underscores the limitations of conventional clinical and functional assessments alone and provides a strong rationale for molecular stratification approaches. Biomarkers capable of reflecting pathway-specific activity such as inflammation-associated miRNAs (e.g., miR-155) or fibrosis-associated miRNAs (e.g., miR-21) may enable discrimination between disease phenotypes, identification of patients at higher risk of progression, and more precise monitoring of therapeutic responses. Incorporating miRNA-based biomarker panels into clinical practice therefore represents a critical step toward personalized management strategies for sulfur mustard-exposed individuals, aligning diagnostic and therapeutic decisions with the underlying molecular drivers of disease.

The elucidation of these pathways translates into tangible clinical opportunities:

Diagnostic and Prognostic Biomarker Panels: Validated panels of miRNAs from serum or urine, building on discoveries like miR-9, miR-143, and others, could serve as objective, non-invasive tools for diagnosing SM-related chronic disease, stratifying disease severity, and predicting the risk of progression (e.g., declining lung function).

Monitoring Disease Activity and Therapy: Serial measurement of these miRNA signatures could allow clinicians to monitor subclinical disease activity and assess the efficacy of new therapeutic interventions in clinical trials more sensitively than traditional pulmonary function tests alone.

Novel Therapeutic Targets: The molecular players identified offer new avenues for intervention:

Anti-fibrotics: Agents inhibiting the TGF- β pathway or specific downstream effectors.

miRNA-Targeted Therapy: Using antagomirs (to inhibit overexpressed miRNAs like miR-21) or miRNA mimics (to restore deficient miRNAs).

Anti-inflammatories and Antioxidants: Targeted therapies to break the cycle of chronic inflammation and oxidative stress, potentially using advanced formulations like nanoparticles for lung delivery [23]

Conclusion

The chronic toxicity of sulfur mustard represents a profound medical challenge, where a brief exposure alters biological fate decades later. Research has progressively moved from descriptive symptomatology to a deep dissection of the molecular continuum that connects acute alkylation to chronic fibrosis and remodeling. The

integration of pathogenesis studies—highlighting TGF- β , TFF1, and oxidative stress—with the innovative field of miRNA biomarker discovery provides a complete and more dynamic picture of this disease process. This evolving understanding, grounded in studies on exposed veterans, not only honors their suffering by seeking to explain it but also illuminates a path forward. Through continued interdisciplinary research,

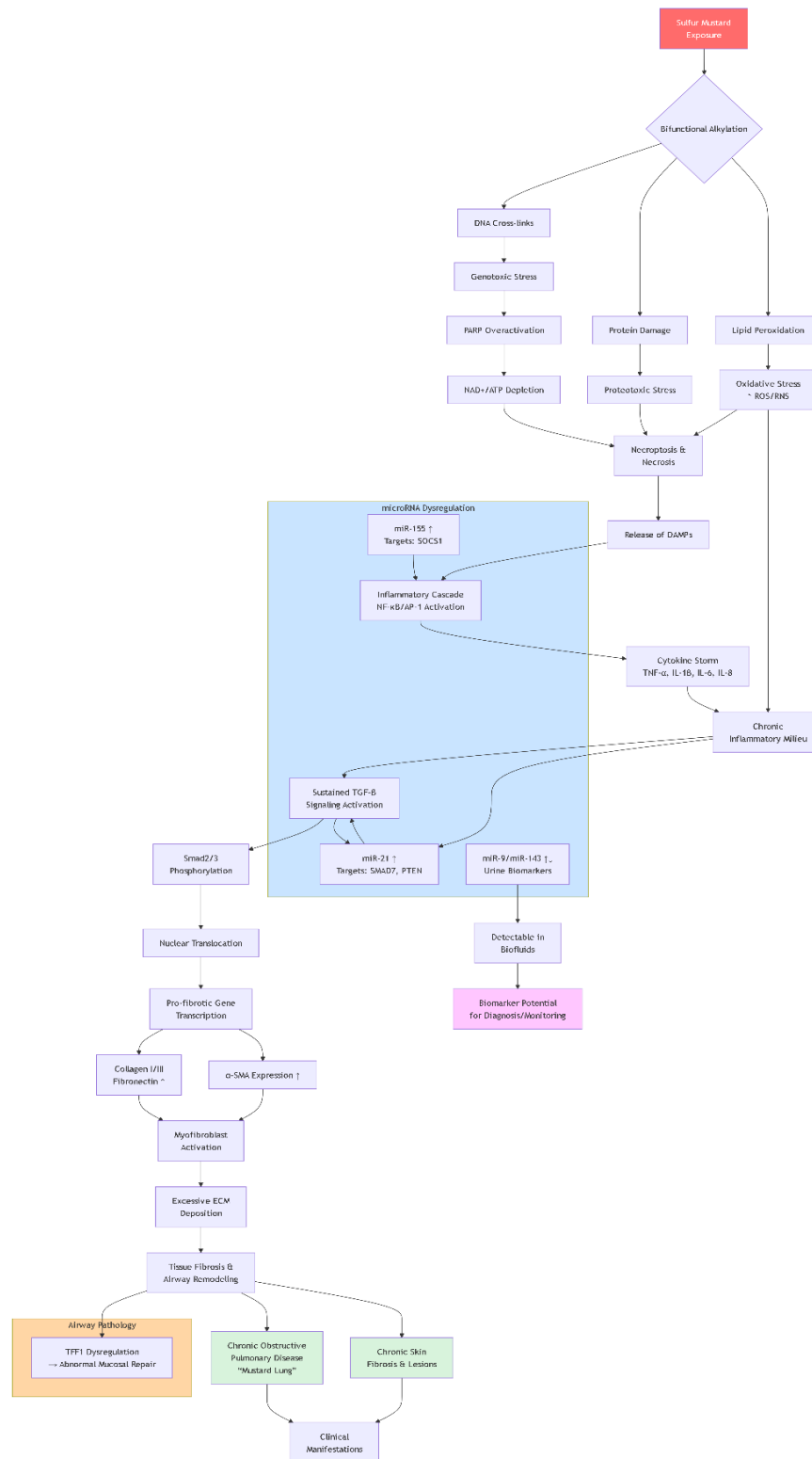


Figure 1. Integrated molecular pathway of sulfur mustard-induced chronic toxicity.

Red: Initial alkylation damage. Blue: Fibrotic transformation via TGF- β /Smad. Light blue box: microRNA dysregulation and biomarker detection. Orange box: TFF1-mediated airway remodeling. Light green: Chronic clinical manifestations. Pink: Translational biomarker potential. Arrows indicate direction of influence or progression.

the goal of translating molecular insights into effective diagnostics and therapies for SM-exposed individuals becomes increasingly attainable.

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