Anti-RNA Polymerase III Antibodies in Systemic Sclerosis

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Abstract

Anti-RNA Polymerase III has been recognized as an important autoantibody in Systemic Sclerosis and it is now included in the 2013 ACR/EULAR classification criteria for Systemic Sclerosis. With this manuscript we attempt to review the current data on anti-RNA polymerase II as it relates to Systemic Sclerosis.

Keywords
Autoantibodies, Systemic Sclerosis, Scleroderma, Anti-RNA Polymerase III, Cancer

1. Introduction

Systemic Sclerosis (SSc) is an autoimmune connective tissue disorder characterized by tissue fibrosis, vascular dysfunction, both structural and functional, early cellular infiltration of affected tissues, with local cytokine and growth factor upregulation as well as B-cell abnormalities resulting in the production of autoantibodies. It affects multiple organ systems, has a peak incidence between 20 - 50 years of age, and it can be chronic and progressive in nature with female predominance [1] [2]. SSc is subdivided based on skin involvement into limited cutaneous Systemic Sclerosis (lcSSc) which involves the skin distal to the knees and elbows, and diffuse cutaneous Systemic Sclerosis (dcSSc) which involves the skin proximal to the knees and the elbows. Less common forms of SSc are sclerosis sine scleroderma, overlap syndromes, and undifferentiated scleroderma. Raynaud’s phenomenon, skin tightness/thickening, myalgias, arthralgias, digital ulcers, pulmonary arterial hypertension (PAH), gastrointestinal symptoms, and renal impairment are some of the disease manifestations of SSc. The etiology of the disease is unknown but believed to be a combination of genetic and environmental factors [3] [4].

SSc is associated with various autoantibodies including anti-centromere antibodies (sensitivity 33%, specific-
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antibody, its association with SSc, and potential future applications of the biomarker. This review seeks to examine past and current literature on the anti-RNAP III antibody, and are often accompanied by anti-RNAP II [17]. Anti-RNAP I ab are believed to localize to nucleolus. RNA Pol I is known to be located in nucleoli, while RNA Pol II and III are found in the nuclei. Finally, immunofluorescence studies were performed on all 57 sera. Characteristic nucleolar and nuclear RNA Pol I staining was observed in 17 patients (7 from group 1, 10 from group 2, and none from group 3). The remaining 40 sera showed only nuclear speckled staining. RNA Pol I is known to be located in nucleoli, while RNA Pol II and III are found in the nuclei. Finally, Okano et al. tested for the presence of anti-RNA Pol III ab in patients with SSc with other autoantibodies. None of these patients were found to have anti-RNA Pol III ab in addition to another autoantibody. Further analysis revealed that of the SSc patients who were positive for Anti-RNAP III ab 45% had dcSSc (compared to 7% with lcSSc). Finally, these patients also had significant skin thickness and were more likely to have scleroderma renal crisis [16].

Due to the time consuming and complicated nature of immunoprecipitation assays and the inconsistency of indirect immunofluorescent (IIF) staining, it was difficult to employ the use of anti-RNA Pol III antibody detection in routine clinical practice. To demonstrate the difficulty with IIF, Yamasaki et al. described autoantibodies from all three RNA polymerases as biomarkers for SSc [15]. Okano et al. took 252 serum samples of patients with SSc of these 161 samples precipitated specific antibodies previously described in SSc. However, the remaining sera from 91 patients produced nuclear, or nucleolar staining, or both towards an unidentified antigen. Of the 91 samples, 57 precipitated proteins that could represent RNA Pol I, II, or III. These were broken down into 3 groups based upon protein precipitation pattern using previously identified molecular weights and structures of RNA polymerases. Group 1 exhibited proteins similar to all RNA Pol I, II, and III (n = 13). While group 2 was likely RNA Pol I, and II (n = 27), and group 3 was suspected to be RNA Pol III (n = 17). To confirm the hypothesis, immunoblotting studies were performed on the sera directed against anti-RNA Pol III. Afterward, immunodepletion studies were conducted on the 3 groups of sera for confirmation. Finally, immunofluorescence studies were performed on all 57 sera. Characteristic nucleolar and nuclear RNA Pol I staining was observed in 17 patients (7 from group 1, 10 from group 2, and none from group 3). The remaining 40 sera showed only nuclear speckled staining. RNA Pol I is known to be located in nucleoli, while RNA Pol II and III are found in the nuclei. Finally, Okano et al. tested for the presence of anti-RNA Pol III ab in patients with SSc with other autoantibodies. None of these patients were found to have anti-RNA Pol III ab in addition to another autoantibody. Further analysis revealed that of the SSc patients who were positive for Anti-RNAP III ab 45% had dcSSc (compared to 7% with lcSSc). Finally, these patients also had significant skin thickness and were more likely to have scleroderma renal crisis [16].

Another antibody, anti-RNA-polymerase III (anti-RNAP III) is now included in the most recent American College of Rheumatology (ACR)/EULAR classification criteria for SSc and it is used to predict disease severity [9]. The prevalence of anti-RNAP III among SSc patients has been estimated from 4% - 22% worldwide with geoethnic variation. Higher frequency is seen in USA (8.3%), England (11.7%), Sweden (22%) and lower frequency is noted in Japan (5%) and France (4%) [10]. More recently, a study in Belgium further supports prior results and notes 7% prevalence of anti-RNAP III ab [11]. Similar to other autoantibodies in SSc, anti-RNAP III has a lower sensitivity, but is highly specific for SSc (sensitivity: 10% - 20%, specificity: 98.8%) [12]. Moreover, it is highly associated with dcSSc, associated with scleroderma renal crisis, and skin changes. More recently, anti-RNAP III has been associated with malignancy in patients with SSc. Moinzadeh et al. examines a cohort of SSc (>2000 patients with SSc) and find significantly increased frequency of cancers among patients with anti-RNAP III compared to ACA and anti-Scl-70 abs. Finally, a temporal association is suggested between onset of SSc and breast cancer. That is, among patients diagnosed with cancer within the first 36 months of SSc onset, a large number of them have anti-RNP (55.3%) compared to ACA (23.5%) and anti-Scl-70 (13.6%) [13]. This will be further elaborated below. This review seeks to examine past and current literature on the anti-RNAP III antibody, its association with SSc, and potential future applications of the biomarker.

2. Discovery and Methodology of Testing

Antibodies to RNA Pol III were first described by Okano et al. by immunoprecipitation in a 1992 abstract [14]. However, before their more definitive paper came out, Kuwana et al. described autoantibodies from all three RNA polymerases as biomarkers for SSc [15]. Okano et al. took 252 serum samples of patients with SSc of these 161 samples precipitated specific antibodies previously described in SSc. However, the remaining sera from 91 patients produced nuclear, or nucleolar staining, or both towards an unidentified antigen. Of the 91 samples, 57 precipitated proteins that could represent RNA Pol I, II, or III. These were broken down into 3 groups based upon protein precipitation pattern using previously identified molecular weights and structures of RNA polymerases. Group 1 exhibited proteins similar to all RNA Pol I, II, and III (n = 13). While group 2 was likely RNA Pol I, and II (n = 27), and group 3 was suspected to be RNA Pol III (n = 17). To confirm the hypothesis, immunoblotting studies were performed on the sera directed against anti-RNA Pol III. Afterward, immunodepletion studies were conducted on the 3 groups of sera for confirmation. Finally, immunofluorescence studies were performed on all 57 sera. Characteristic nucleolar and nuclear RNA Pol I staining was observed in 17 patients (7 from group 1, 10 from group 2, and none from group 3). The remaining 40 sera showed only nuclear speckled staining. RNA Pol I is known to be located in nucleoli, while RNA Pol II and III are found in the nuclei. Finally, Okano et al. tested for the presence of anti-RNA Pol III ab in patients with SSc with other autoantibodies. None of these patients were found to have anti-RNA Pol III ab in addition to another autoantibody. Further analysis revealed that of the SSc patients who were positive for Anti-RNAP III ab 45% had dcSSc (compared to 7% with lcSSc). Finally, these patients also had significant skin thickness and were more likely to have scleroderma renal crisis [16].

Due to the time consuming and complicated nature of immunoprecipitation assays and the inconsistency of indirect immunofluorescent (IIF) staining, it was difficult to employ the use of anti-RNA Pol III antibody detection in routine clinical practice. To demonstrate the difficulty with IIF, Yamasaki et al. tested three methods of RNAP III detection using 18 samples of Anti RNAP I/III samples: 1) sera were tested using immunofluorescent antinuclear antibodies on HEp-2 cell slides; 2) by anti-RNAP III ELISA; and 3) by IP assay using 35S-labeled K562 cell extract. Nucleolar staining of anti-RNAP antibody IP-positive sera was confirmed by double staining with antifibrillarin monoclonal antibodies. It was noted that anti-RNAP I almost always coexists with anti-RNAP III ab, and are often accompanied by anti-RNAP II [17]. Anti-RNAP I ab are believed to localize to nucle-
leoli. If this were the case, then IIF could be used as a screening test prior to anti-RNAP III ELISA. All 18 samples showed nuclear speckled/homogeneous staining, but nucleolar staining was clearly present only 42% of the time, while 26% was not obviously present, and 32% of the time it was unrecognizable, making the majority of nucleolar staining samples unclear to unrecognizable. Thus, detection using enzyme-linked immunosorbent assay (ELISA) was developed [18]. However, before an ELISA could be developed an RNA Pol III epitope needed to be identified.

3. Anti-RNA Pol III Epitope

Kuwana et al. identified an RNA Pol III immunodominant epitope and developed an ELISA in 2002. RNA Pol I and III are multiprotein complexes consisting of >12 subunits, thus the development of a subunit-specific assay required recognition of the antigenic subunit recognized by anti-RNAP I/III positive sera. Prior investigation revealed RNAP III-specific 62-kd and 155-kd subunits are recognized by nearly all SSc sera containing anti-RNAP I/III abs, which corresponded to RNAP III subunits RPC62 and RPC155 [19]-[21]. cDNA which encodes the nucleotide sequences for the complete open-reading frames were then identified [20] [22]. Afterward, Kuwana et al. then further examined the epitopes using a series of recombinant fragments expressed in Escherichia coli. These recombinant fragments were used to express the major epitope as antigen, which led to the development of an ELISA for detection of anti-RNAP I/III antibodies.

4. Pathogenesis

To date, the mechanism in which anti-RNA Pol III antibodies cause disease remains unclear. Based on the epitope described above, the subunits are highly conserved throughout multiple organisms (yeast, bacteria, mammalian cells), and it has been suggested that molecular mimicry may lead to the production of antibodies against RNA Pol III [21].

5. RNA Pol III Ab Associations

5.1. Malignancy

Cancer has been associated with SSc ranging from 3.6% - 10.7% in patients with SSc and 75% compared to the general population [23] [24]. Malignancy has been noted more frequently with dcSSc compared to lcSSc [13]. Prior studies have not found significant relationship between ACA and anti-Scl-70 antibodies in patients with SSc and malignancy. However, more recent investigation has noted association with SSc patients with RNA Pol III ab and malignancy [25] [26]. Wooten in a 2008 review article describes lung cancer as the major malignancy, followed by breast cancer, and then gastrointestinal cancers (esophageal (6.2%) being most common), urogenital, lymphomas and other hematologic cancers [24]. More recently, Bonifaze et al. further confirmed SSc and cancer risk [23] again noting the strong association with lung cancer, but did not show an association with breast cancer. Most recently, Moinzadeh et al. conducted the largest population study to date on SSc and cancer (n=2177 with SSc, n=154 pts with SSc and cancer) and found increased frequency of breast cancer (42.2%) then lung cancer (10.4%), and hematological cancers (12.3%) and GI or gynecological (11%). The cohort was 85% female and 63% had lcSSc. Abs to ACAs, anti-Scl-70 and Anti RNP ab were measured and found to contain 26%, 18.2%, and 26.6% respectively. They noted a statistically significant higher frequency of malignancy in patients with anti-RNP abs than those without cancers (26.6% vs. 12.2% respectively P < 0.001), while no statistically significant differences were noted in ACA and anti-Scl-70 abs.

5.2. Temporal Relationship

Investigation into the timing of malignancy diagnosis with respect to SSc diagnosis has yielded some associations of interest. Pulmonary cancer has been associated with long standing SSc > 5 years from dx of SSc and closely associated with pulmonary fibrosis, while breast cancer appears to have a closer temporal relationship between onset of SSc and cancer [23] [27]. Launay et al. reported a less than 12 month duration between onset of breast cancer and diagnosis of systemic sclerosis [28]. Scope noted 75% of reported breast cancer cases and SSc were diagnosed within 3 years of each other [29]. Moinzadeh et al. noted a statistically significant number of patients with SSc and anti-RNP III abs were diagnosed with cancer (again breast cancer most common at
46%) within the first 3 years, and had nearly 6x higher odds of developing cancer in the first 3 years compared to patients without anti-RNAP abs. They found no significant cancer development with patients from 36 - 120 mo after SSc onset. This temporal relationship between SSc and malignancy suggests the possibility of SSc representing a paraneoplastic syndrome in which the immune system is responding to malignancy. There has been implication of abnormal RNAP activity in cancer cells from breast and lung carcinomas as well as fibroblasts transformed by polyomavirus. Thus, it has been suggested that repression of tumor suppressor genes p53 and retinoblastoma and/or activation of oncogene product c-Myc may lead to enhanced RNAP activity. Although, no biologic basis for an association between specific autoantibody subtypes against nucleolar RNA associated proteins and malignancy has been established to date. However, the presence of anti-RNAP ab may initiate an anti-tumor immune response, which in the appropriate setting may cross-react against specific host tissue resulting in damage [30]-[34].

6. Conclusion

Anti-RNAP III antibody is highly specific but not sensitive for SSc. It is associated with dcSSc and often seen with skin thickening and renal crisis. Assays for detection of the antibody have improved from immunoprecipitation, to discovering the inability to use IIF, to now commercially available ELISA. There appears to be an association with SSc and malignancy and some evidence implicating that anti-RNAP III is elevated in these patients. Anti-RNAP III is, to date, the only antibody in SSc to show a significant relationship to malignancy and temporal relationship with breast cancer. However, there is conflicting data on whether breast cancer is associated with SSc. More investigation into the biologic underpinnings between anti-RNAP III and malignancy should be explored. Individuals with SSc who have anti-RNAP III ab and are at high risk for malignancy, may warrant appropriate screening.

References


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