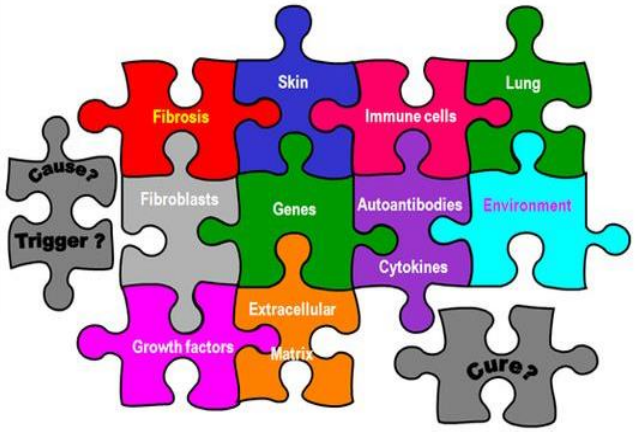


The relationship between WNT signaling activity and organ attitudes in scleroderma disease sub-groups

Ayşe Koçak Sezgin, PhD

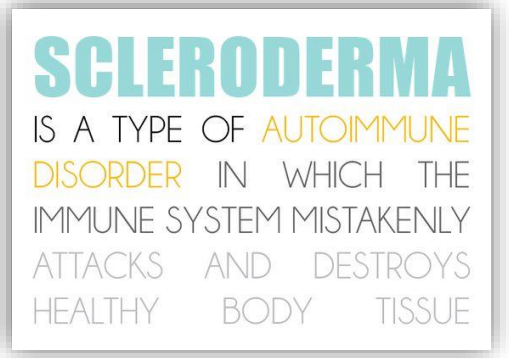
Postdoctoral reseacher

Dokuz Eylul University

28.10.19

Antalya

SCLERODERMA



- Systemic sclerosis or scleroderma (SSc) is a complex disease with three main clinical features: **excessive chronic matrix (ECM) accumulation (FIBROSIS)**, **vascular damage** and **inflammation autoimmunity.**

- Although the pathogenesis is unclear, SSc is characterized by abnormal reshaping of connective tissues in the skin and internal organs, due to the overproduction of ECM, in particular that of collagen, by fibroblasts.

Ayse Kocak et al.: Effects of epigallocatechin-3-gallate (EGCG) on a scleroderma model of fibrosis (2018) TJB. https://doi.org/10.1515/tjb-2017-0185

Epidemiology

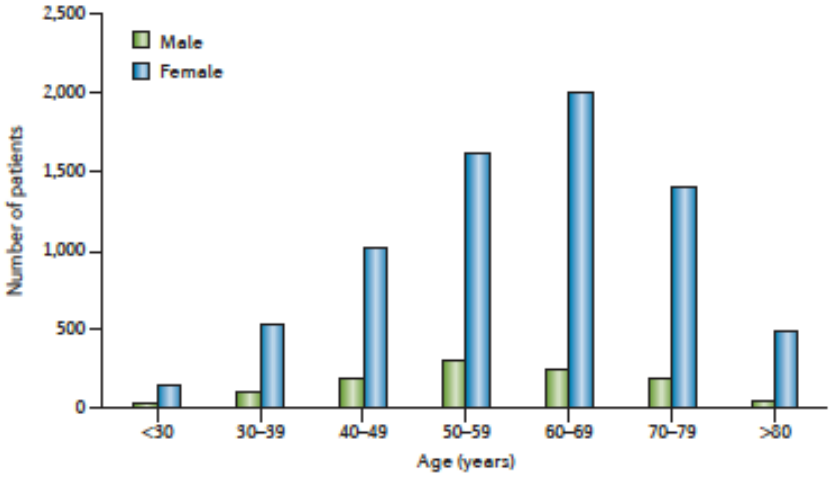
- Peak age range: 35-64
    - Younger age in women and with diffuse disease.
- Female:Male = 3:1
    - 8:1 in child bearing years
- Incidence: 20 per million in a year in US
- Prevalence: 240 per million in US.

Major cause of death:

1) renal involvement

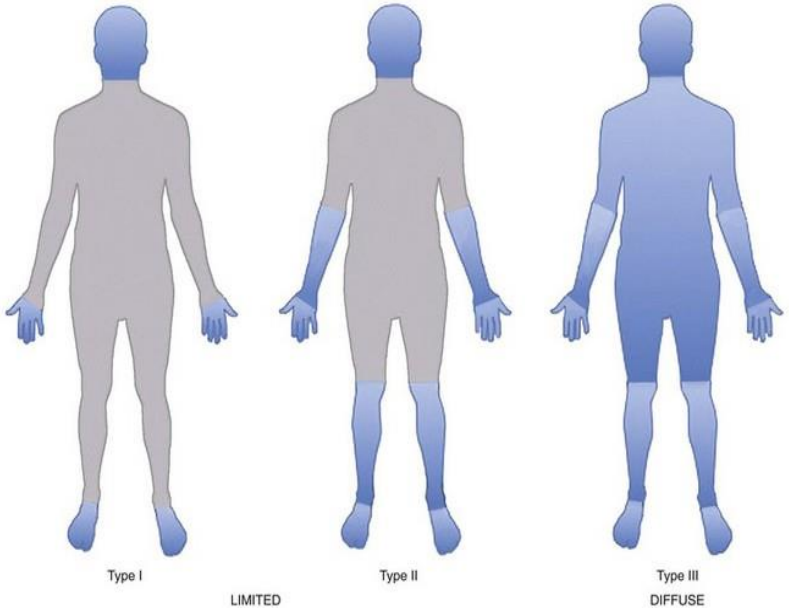
2) cardiac involvement

3) pulmonary involvement



Yannick Allanore et al. Systemic sclerosis.Nature Reviews Disease Primers (2015) volume1, Article number: 15002.

Forms of Scleroderma



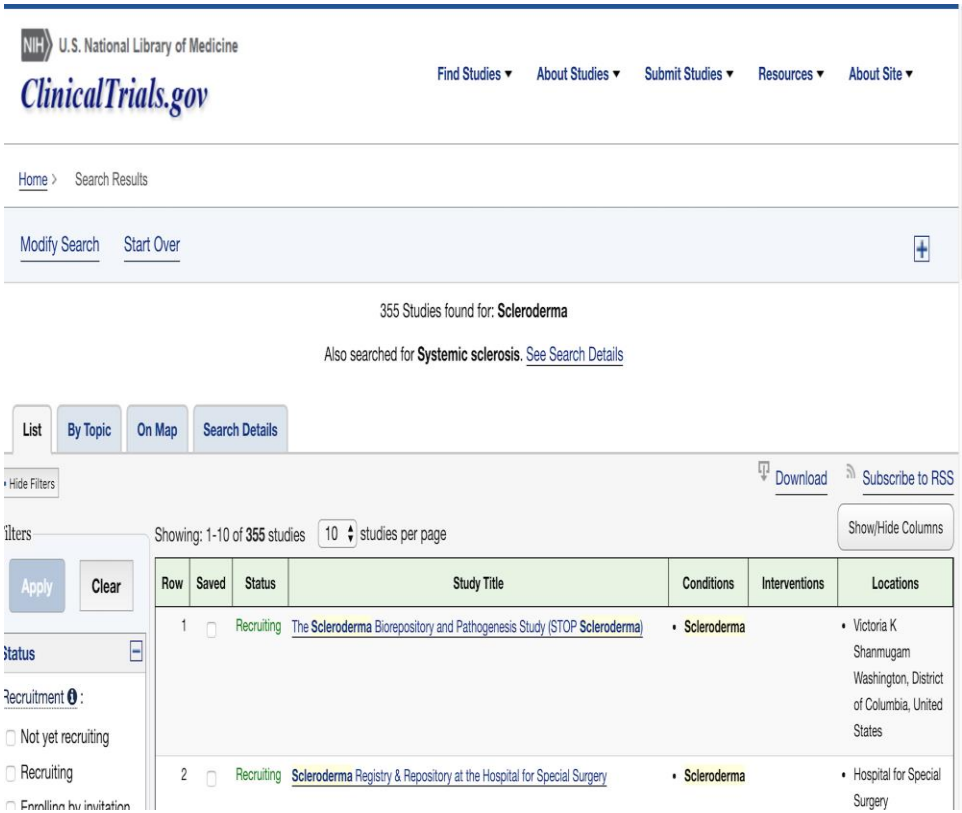
- **Limited Scleroderma**
  - Proximal skin thickening
  - Can involve perioral skin thickening (pursing of lips)
  - Less organ involvement
  - Seen in CREST syndrome
  - Isolated pulmonary hypertension can occur
- **Diffuse Scleroderma**
  - Skin thickening proximal and involving the trunk
  - More likely to have organ involvement
  - Pulmonary fibrosis and Renal Crisis are more common.

Bhattacharyya, S., Wei, J. & Varga, J. Understanding fibrosis in systemic sclerosis: shifting paradigms, emerging opportunities. Nat. Rev. Rheumatol. 8, 42–54 (2012).

Treatment

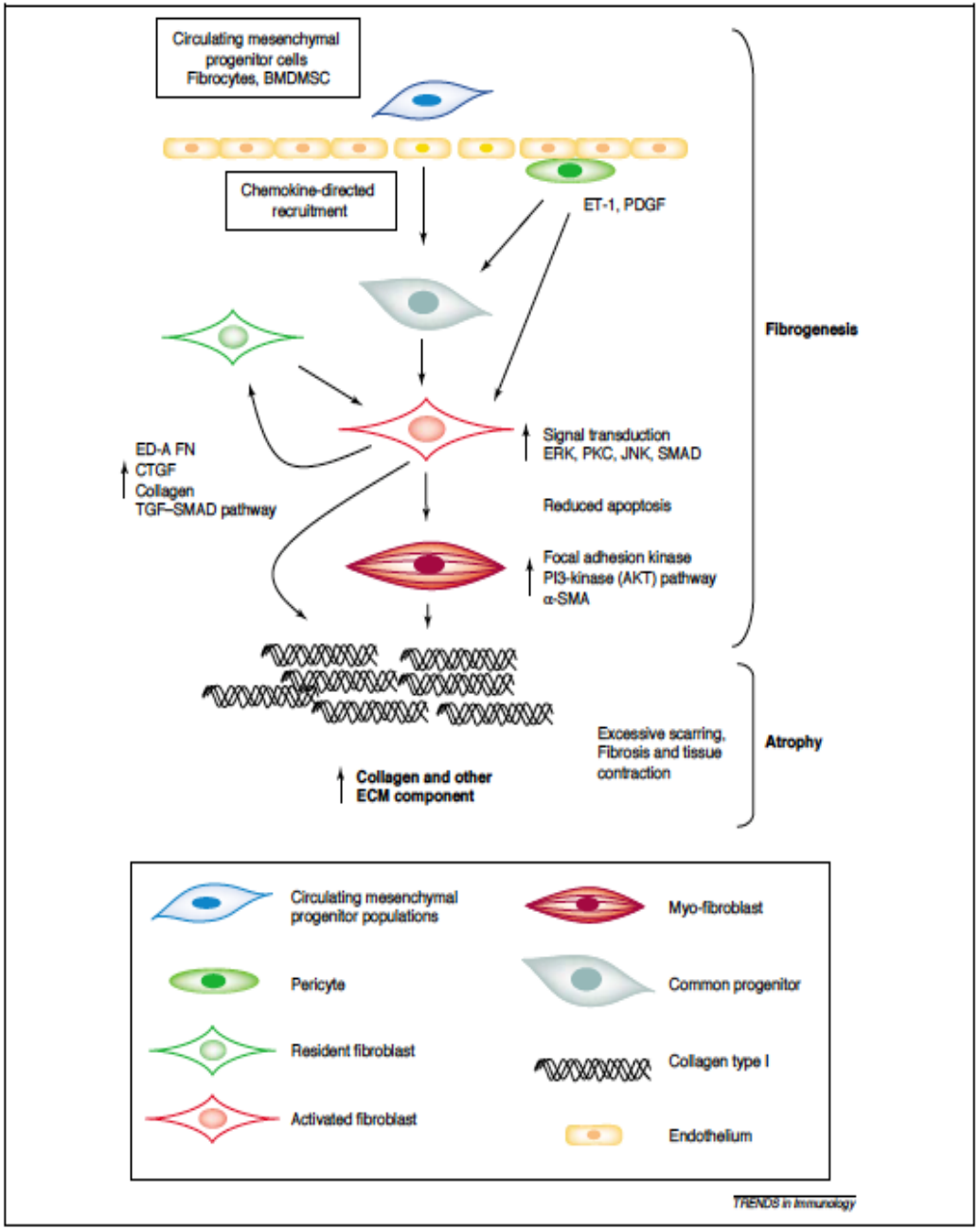
Scleroderma does not have molecular pathway-based treatment option.
Some clinical trials are being conducted.

DRUG	TARGET	OUTCOME OF TRIAL
Fresolimumab	TGF-β	Phase 2, improvement in mRSS and gene biomarker in skin
Abituzumab	αv integrin	Phase 2, enrolling
SAR100842	LPA_1 receptor	Phase 2, trend toward improvement in mRSS
Tocilizumab	IL-6 receptor	Phase 2, trend toward improvement in mRSS 48 weeks; possibly slower decline in FVC
Pirfenidone	Fibroblast proliferation	Phase 2, acceptable safety profile
Nintedanib	Inhibits multiple receptor tyrosine kinases and non-receptor tyrosine kinases	Phase 3, enrolling
Abatacept	Fusion protein to CTLA-4	Phase 2, enrolling
Rilonacept	IL-1	Phase 2, enrolling



www.clinicaltrials.gov.tr, 08.01.2018

MOLECULAR MECHANISM OF SCLERODERMA

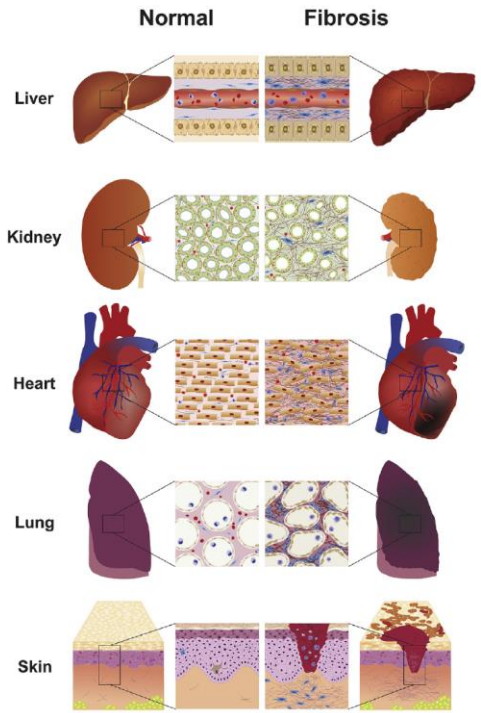


Fibrogenesis (Fibrosis) in SSc:
-TGF-beta pathway is activated
-ERK, PKC, JNK, SMAD signaling ↑
-PI3-kinase (AKT) pathway ↑
-α-SMA ↑
-Myofibroblasts ↑
-Collagen ↑
-ECM components ↑

WNT family ?

Fibrosis and Scleroderma

- Fibrosis is a reactive process that is majorly characterised by the formation and deposition of excess connective tissue resulting in progressive remodeling in nearly all tissues and organs.

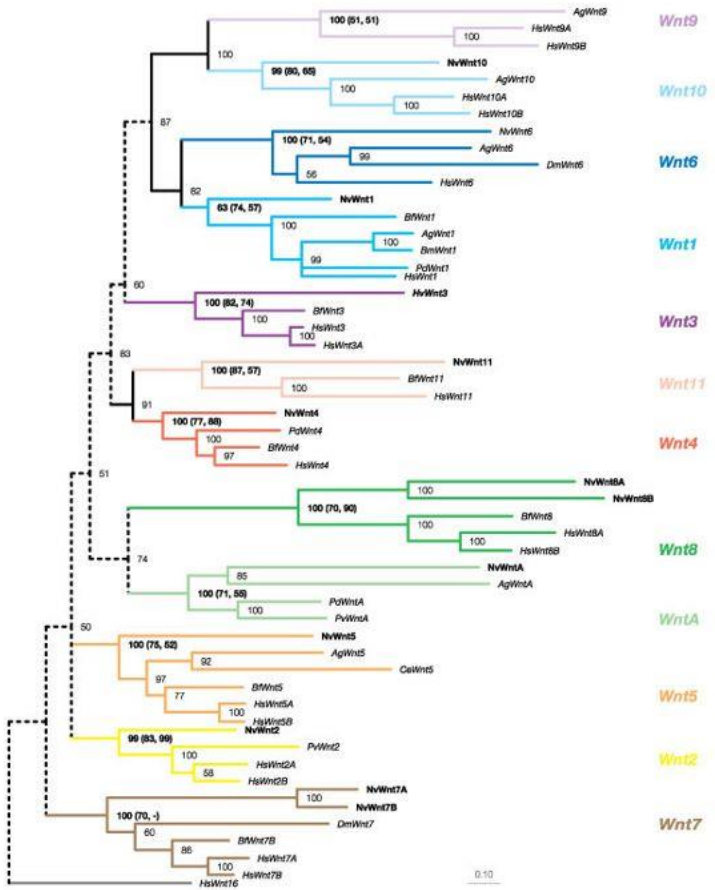


Injurious processes lead to organ damage, inflammation, and fibrosis **in liver, kidney, lung, heart and skin.**

Hallmarks of fibrotic processes shared by all of these tissues include the excessive production of **cytokines, chemokines, growth factors, extracellular matrix (ECM) proteins** as well as loss of normal organ structure and function.

Weiskirchen, R., Molecular Aspects of Medicine (2018),https://doi.org/10.1016/j.mam.2018.06.003

Wnt Family function in fibrosis, Scleroderma



Wnt/β-catenin signaling play a role in severe fibrotic diseases, such as pulmonary fibrosis, liver fibrosis, skin fibrosis and renal fibrosis.

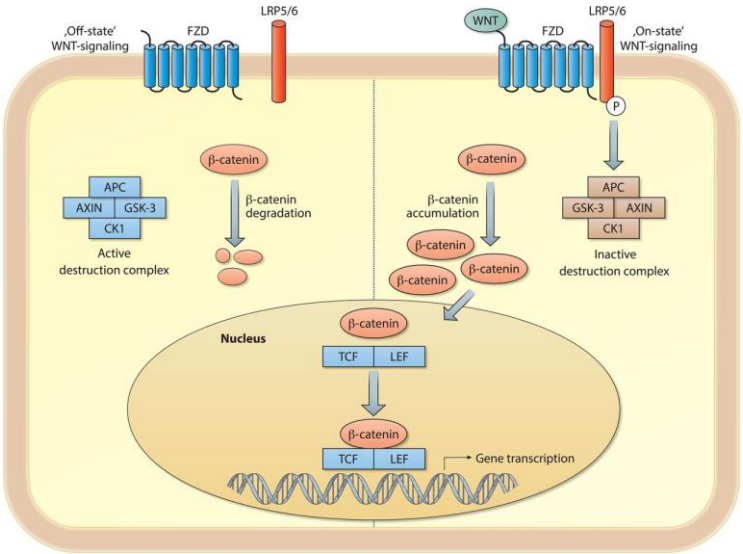
Microarray gene expression in skin of Tight-skin (Tsk) mouse (an animal model of SSc) showed increased mRNA levels of several genes, including Wnt2, Wnt3a, Wnt9a, Wnt10b and Wnt11.

But there is no data in literature about WNT signaling family and scleroderma patient & scleroderma organ involvement .

BAYLE J, FITCH J, JACOBSEN K, KUMAR R, LAFYATIS R, LEMAIRE R: Increased expression of Wnt2 and SFRP4 in Tsk mouse skin: role of Wnt signaling in altered dermal fibrillin deposition and systemi sclerosis. J Invest Dermatol 128: 871-881, 2008.
Kocak A et al. Molecular Mechanisms of Scleroderma and Fibrosis (2018) http://dx.doi.org/10.20431/2349-0365.0501007

Wnt Family function in fibrosis, Scleroderma

Moreover, **transgenic mice expressing Wnt-10b showed progressive loss of subcutaneous adipose tissue accompanied by dermal fibrosis**, and Wnt-10b infection of normal fibroblasts and preadipocytes resulted in blockade of adipogenesis and up-regulation of fibrotic gene expression, suggesting that Wnt-10b switches differentiation of mesenchymal cells toward myofibroblasts by inducing a fibrogenic transcriptional program while suppressing adipogenesis



WEI J, BHATTACHARYYA S, TOURTELLOTTE WG, VARGA J: Fibrosis in systemic sclerosis: emerging concepts and implications for targeted therapy. Autoimmun Rev 10: 267-275, 2011.

Aim:

- We aimed to show the relationship of WNT gene family and antagonists in development of SSC subtypes of disease and different organ involvement.

Materials and Methods:

- The study included **85 patients** with SSC and **77 controls**.
- The gene expressions & protein levels of the WNT family and antagonists were analyzed from blood samples.
- The **qPCR method** was used for WNT gene expression levels. WNT antagonists protein levels were determined by **ELISA** method.
- The relationship between these parameters and disease stage, type and organ involvement were evaluated.

Results

Specification(s)	Mean±SD Median (Min-Max)
Age	54,82±12,069 58 (22-80)
Gender	n (%)
Female	76 (%89,4)
Male	6 (%10,6)
Clinical subtypes	n (%)
Limited	53 (%63)
Generalized	32 (%37)
Pulmonary arteriel hypertension (PAH)	n (%)
PAH (+)	6 (%7,1)
PAH (-)	76 (%92,9)

Table 1. Classifications of scleroderma patients

WNT gene family (ΔΔCT) (FOLD)	CONTROL	SCLERODERMA	p value
WNT-1	1	1,79	**0,030***
WNT-10b	1	1,77	**0,002***
WNT-2b13	1	0,99	0,775
WNT-2	1	46,68	**0,007***
WNT-3a	1	2,77	0,064
WNT-4	1	1,02	0,558
WNT-6	1	2,6	**0,000***
WNT-7a	1	1,14	0,389
WNT-7b	1	0,90	0,305
WNT-8a	1	3,7	0,056
WNT-9b	1	1,10	0,959
WNT-9a	ND	ND	ND
WNT-10a	1	1,19	0,847
WNT-16	1	1,06	0,091
AXIN-2	1	0,23	**0,012***
PROTEIN LEVELS			
DKK-1 (ng/mL)	32,88	9,88	**0,015***
Kremen (ng/mL)	1438	630	**0,000***

ND: Not detected, *p<o.o5

WNT-10b, WNT-2, WNT-6 and WNT-1 significantly increased in Scc.

DKK-1, Kremen, Axin-2 are significantly decreased in Scc.

Data not published

WNT gene family (ΔΔCT) (FOLD)	LIMITED SCLERODERMA	GENERALIZED (DIFFUSE) SCLERODERMA	p value
WNT-1	6,4	8,8	0,463
WNT-10b	14,2	9,5	0,615
WNT-2b13	4,3	3,4	0,287
WNT-2	8,1	8,7	0,964
WNT-3a	6,14	37,2	**0,001***
WNT-4	12,0	4,27	0,170
WNT-6	2,1	3,9	0,144
WNT-7a	79,1	61,9	0,654
WNT-7b	32,2	33,1	0,897
WNT-8a	15,4	11,8	0,118
WNT-9b	19,4	16,16	0,464
WNT-9a	ND	ND	ND
WNT-10a	21,3	44,6	**0,010***
WNT-16	9,96	10,41	0,985
AXIN-2	5,7	11,8	0,104
PROTEIN LEVELS			
DKK-1 (ng/mL)	8,9	10,46	0,216
Kremen (ng/mL)	2829,5	2864,8	0,289

ND: Not detected, *p<o.o5

Wnt-3a, Wnt-10a expression levels significantly increased in generalized scleroderma.

WNT gene family (ΔΔCT) (FOLD)	PAH (+) SCLERODERMA	PAH (-) SCLERODERMA	p value
WNT-1	21,9	6,2	**0,023***
WNT-10b	1,35	13,34	0,139
WNT-2b13	6,04	3,85	0,809
WNT-2	4135,47	588,01	**0,006***
WNT-3a	103	305,7	0,715
WNT-4	13,54	77,16	0,471
WNT-6	37,1	32,22	0,232
WNT-7a	3,13	3,42	0,135
WNT-7b	10,06	22,81	0,831
WNT-8a	3,13	3,6	0,580
WNT-9b	10,06	22,8	0,289
WNT-9a	ND	ND	ND
WNT-10a	9,77	31,6	0,300
WNT-16	4,23	10,58	0,294
AXIN-2	29,7	6,37	**0,005***
PROTEIN LEVELS			
DKK-1 (ng/mL)	7,8	9,98	0,253
Kremen (ng/mL)	2153	2895	0,273

ND: Not detected, *p<o.o5

Wnt-1, Wnt-2, Axin-2 expression levels increased in PAH (+) scleroderma patients.

WNT gene family (ΔΔCT) (FOLD)	Rodnan skin score (mRSS) (r)	p VALUE
WNT-1	0,14	0,900
WNT-10b	-0,180	0,099
WNT-2b13	0,25	0,822
WNT-2	0,317	**0,003 ***
WNT-3a	-0,34	0,756
WNT-4	0,013	0,905
WNT-6	0,159	0,149
WNT-7a	0,046	0,678
WNT-7b	-0,083	0,451
WNT-8a	-0,141	0,863
WNT-9b	0,019	0,993
WNT-9a	ND	ND
WNT-10a	0,09	0,935
WNT-16	0,107	0,330
AXIN-2	0,036	0,744
PROTEIN LEVELS		
DKK-1 (ng/mL)	0,009	0,962
Kremen (ng/mL)	0,083	0,449

ND: Not detected, *p<o.o5

Wnt-2 and Rodnan Skin Score had positive correlations

WNT gene family (ΔΔCT) (FOLD)	DISEASE PROGRESSION SCALE (r)	p value
WNT-1	0,053	0,635
WNT-10b	0,194	0,076
WNT-2b13	0,032	0,771
WNT-2	0,002	0,985
WNT-3a	0,046	0,675
WNT-4	0,045	0,689
WNT-6	0,069	0,537
WNT-7a	-0,074	0,508
WNT-7b	-0,95	0,388
WNT-8a	0,145	0,780
WNT-9b	0,042	0,711
WNT-9a	TE	
WNT-10a	0,050	0,650
WNT-16	0,026	0,817
AXIN-2	-0,024	0,830
PROTEIN LEVELS		
DKK-1 (ng/mL)	-0,187	0,297
Kremen (ng/mL)	-0,003	0,978

ND: Not detected, *p<o.o5

There is no significance between disease progression scale and Wnt family genes.

WNT gene family (ΔΔCT) (FOLD)	GIS score (r)	p value
WNT-1	0,37	**0,007***
WNT-10b	0,19	0,880
WNT-2b13	0,209	0,098
WNT-2	0,286	**0,022***
WNT-3a	-0,79	0,536
WNT-4	0,424	**0,001***
WNT-6	0,148	0,248
WNT-7a	0,114	0,371
WNT-7b	0,086	0,500
WNT-8a	0,350	**0,005***
WNT-9b	0,323	**0,011***
WNT-9a	ND	ND
WNT-10a	0,128	0,312
WNT-16	0,032	0,801
AXIN-2	-0,070	0,581
PROTEIN LEVELS		
DKK-1 (ng/mL)	-0,069	0,742
Kremen (ng/mL)	-0,112	0,378

ND: Not detected, *p<o.o5

Wnt-1, Wnt-2, Wnt-4, Wnt-8a, Wnt-9b gene expressions and GIS scores had positive corelations.

Conclusion 1. Significant increase in WNT-1, WNT-10b, WNT-2, WNT-6 genes were increased in scleroderma group and Axin-2, DKK-1, Kremen were decreased. They were used for prognostic markers. It may be helpful in choosing the treatment regimen or approach or predicting its effects.

Conclusion 2. WNT-3a and WNT-10a is important for the classification of the disease.

Conclusion 3. WNT-1, WNT-2 and AXIN-2 gene in PAH (+) scleroderma patients may be important in the of the disease classifications.

Conclusion 4. No significant difference was found between the disease severity scale and WNT gene family of scleroderma patients.

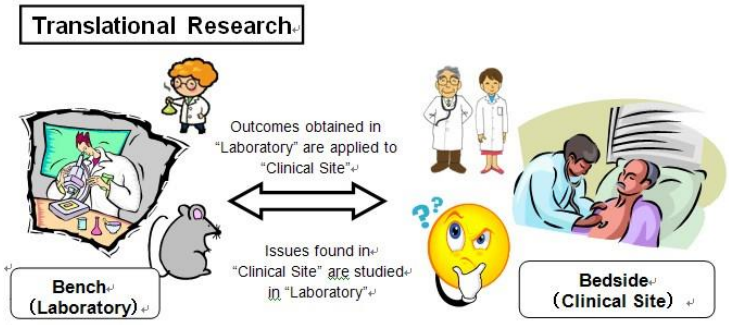
Conclusion 5. WNT-1, WNT-2, WNT-4, WNT-8a, WNT-9b in patients with scleroderma may lead to gastrointestinal involvement.

Conclusion 6. **WNT-1, WNT-2** gene expression is high in scleroderma analyzes, indicating that it is a strong **biomarker** candidate for scleroderma. Thus, optimization of treatment methods can be realized by developing targeted therapies to the WNT pathway.

Conclusion 7. The evaluated WNT gene family and antagonists may be promising as new prognostic markers in the treatment of scleroderma by investigating treatment approaches with different mechanisms of action for disease staging and organ involvement.

Translational Implications & Future Aspects

- Important --> DEFINE THE PATHWAY of FIBROSIS and SCLERODERMA
- Extracellular matrix structure, signaling pathways are related a lot of diseases! (orijin –> FIBROSIS)
- The continuous development and application of the technologies and methods will make it possible to identify and discover more common mechanisms, targets and drugs in fibrosis.
- **From bench to bedside** approach is IMPORTANT! Wnt signaling family is used for this approach. Especially WNT-1 and WNT-2!



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