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Research Article

Increased radiosensitivity and impaired DNA repair in patients with STAT3-LOF and ZNF341 deficiency, potentially contributing to malignant transformations

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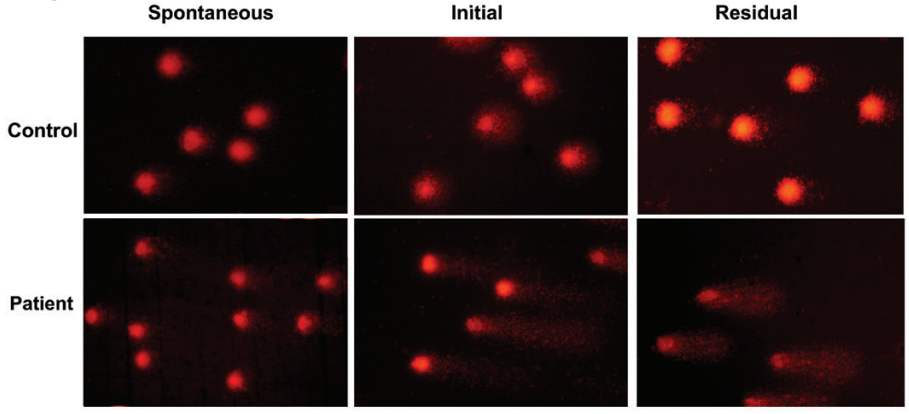
Abstract

STAT3 plays an important role in various complex and sometimes contradictory pathways such as proliferation, differentiation, migration, inflammation, and apoptosis. The transcriptional activity of the STAT3 gene is controlled by a transcription factor called ZNF341. There is insufficient data on radiation sensitivity and post-radiation DNA repair in STAT3- loss-of-function (LOF) patients. We aimed to investigate the radiosensitivity in patients with STAT3-LOF and ZNF341 deficiency. Twelve patients with STAT3-LOF and four ZNF341-deficiency patients were recruited from three clinical immunology centers in Turkey and evaluated for radiosensitivity by the Comet assay, comparing to 14 age- and sex-matched healthy controls. The tail length (TL) (μm), percentage of DNA in the tail (TDNA%), and olive tail moment (OTM) (arbitrary units) were evaluated at the same time for baseline (spontaneous), initial (immediately after 2 Gy irradiation), and recovery (2 h after irradiation) periods by using a computerized image-analysis system, estimating DNA damage. Except for a patient with ZNF341 deficiency who developed nasal cell primitive neuroendocrine tumor and papillary thyroid cancer during the follow-up, there was no cancer in both groups. During the recovery period of irradiation, TL, TDNA%, and OTM values of healthy controls decreased rapidly toward the baseline, while these values of patients with STAT3-LOF and ZNF341 deficiency continued to increase, implying impaired DNA repair mechanisms. Increased radiosensitivity and impaired DNA repair were demonstrated in patients diagnosed with STAT3-LOF and ZNF341 deficiency, potentially explaining the susceptibility to malignant transformation.

Graphical Abstract



Keywords: STAT3-LOF, ZNF-341 deficiency, radiosensitivity, DNA repair, Comet assay

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Abbreviations: DNA, deoxyribonucleic acid; STAT3, signal transducer and activator of transcription 3; STAT3-LOF, STAT3-loss of function; ZNF341, zinc finger 341; TL, tail length; TDNA%, tail DNA (%); OTM, olive tail moment; AT, ataxia telangiectasia; SD, standard deviation; GOF, gain of function

Introduction

The transcription factor signal transducer and activator of transcription 3 (STAT3) mediate the actions of many cytokines involved in mounting innate and adaptive immune responses, whereby drive many critical cellular processes, including growth and apoptosis [1, 2]. Dominant-negative, heterozygous STAT3-loss-of-function (LOF) germline mutations cause autosomal dominant hyper immunoglobulin (Ig) E syndrome (HIES) characterized by eczematoid dermatitis, recurrent skin and sinopulmonary infections, and high IgE levels [1–3]. STAT3-HIES patients usually display normal serum IgE levels, except for IgE, and most of them exhibit insufficient antigen-specific antibody responses after immunization with T-cell-dependent antigens [4]. STAT3 regulates DNA damage response signaling pathway by modifying ataxia-telangiectasia mutated-Serine/threonine-protein kinase Chk1, and dysregulated STAT3 can become a potent oncogene that licenses many human cancers' development [5, 6].

The zinc finger (ZNF) domains are small protein motifs that coordinate various biological functions, including gene transcription, translation, mRNA trafficking, cytoskeletal organization, protein folding, and chromatin remodeling [7]. ZNF341 is a transcription factor that controls the transcription of the STAT3 gene. Biallelic nonsense mutations encoding truncated forms of ZNF341 are responsible for the STAT3-like hyper-IgE phenotype [8, 9].

Although the role of STAT3 in the process of DNA repair has been well defined, there is insufficient data on DNA repair status in patients diagnosed with STAT3-LOF disease. We recently reported impaired DNA repair processing in a patient with ZNF341 deficiency, highlighting the role of STAT3 in cancer development, and questioned whether the exact mechanism could also be linked to STAT3-LOF disease [10].

The Comet assay was first developed by Ostling and Johansson [11]. It is a technique for measuring DNA-strand breaks and repairing at the level of a single cell [12, 13]. In the last two decades, Comet assay has become one of the most common tests to evaluate DNA damage and repair [14].

Herein, we aimed to investigate radiation sensitivity and DNA repair status after irradiation in patients with STAT3-LOF and ZNF341 deficiency by Comet assay.

Materials and methods

Patients and controls

Twelve patients with STAT3-LOF and four with ZNF341 deficiency genetically confirmed from three tertiary clinical immunology centers in Turkey were included in the study. The control group consisted of 14 healthy individuals who were mates in gender and age and did not have cancer in themselves and their relatives.

For the positive control of the comet test, three patients with Ataxia telangiectasia (AT) and two healthy controls were included in the study.

Comet assay

The comet assay was applied to peripheral blood leucocytes of the patients to determine DNA repair problems causing radiosensitivity. The alkaline version of the comet assay was used as it allows to detection of DNA damages induced by clinically relevant doses of radiation [15]. White blood cells of age- and sex-matched healthy blood donors were used as control. The extents of spontaneous (0 Gy), initial (immediately after 2 Gy irradiation), and residual (2 h after irradiation) DNA damage were evaluated by quantifying the tail length (TL) (μm), the percentage of DNA in the tail (TDNA%), and olive tail moment (OTM) (arbitrary units) parameters under an epifluorescence microscope using a computer-based image analysis system (Kameram Komet Module, Micro System Ltd., Turkey). Experiments were performed in triplicate for each subject.

Statistical analysis

Categorical data were expressed as frequencies and percentages, and continuous data as mean ± standard deviation (SD). The size of the differences between the group means was calculated by considering the effect size (ES) of Cohen's d. The ES values were evaluated as 0.2 is low, 0.5 is medium, 0.8 is high, and 1.3 is very high. Statistical analysis was performed using the statistical package SPSS 23 (IBM corp.).

Results

The demographic and clinical features of the patients

STAT3-LOF

The female/male ratio of the patients was 1.4 (7/5), and the median age was 16.1 years (min: 3.9 to max: 39.9 years). No history of malignancy was present in all cases with STAT3-LOF or in their first-degree relatives. None of them had a chronic viral infection. The clinical and laboratory features of the patients were summarized in Supplementary Table S1. It was observed that the effect size of the difference between the mean initial TDNA% measurement of STAT3-LOF and control groups was moderate, and the size of the difference between the groups in other parameters was thoroughly high and vastly significant (Table 1, Figure 1).

ZNF341 deficiency

Four previously reported ZNF341-deficient patients from two distinct families were included in the study [8, 9]. Three biological siblings belonging to one family, aged 21.6, 34.8, and 40.2 years, had a mutation in exon 8 of the *ZNF341* gene (c.1156C>T, p.R386*), and the other patient, aged 27, from a different family, had c.1062delG deletion (p.K355fs). These two variants were detected by whole-exome sequencing, and there was no additional variant contributing to the defective DNA repairing. Only the oldest patient developed nasal cell primitive neuroendocrine tumor and secondary papillary thyroid cancer [10]. Malignancy in the family history was not present. None of them had a chronic viral infection that could lead to malignancy. The clinical and laboratory characteristics of the patients are shown in Supplementary Table S1.

Table 1: Comet assay results of patients with STAT3- LOF (n = 12) and controls (n = 10)

		Number of tested cells		Mean ± standard deviation		ES
		STAT3-LOF	Controls	STAT3-LOF	Controls	
TL	Spontaneous	1200	1000	12.4 ± 4.5	8.7 ± 3.1	0.97
	Initial	1200	1000	37.3 ± 13.4	19.5 ± 5.9	>1.30
	Residual	1200	1000	50.5 ± 18.6	16.0 ± 5.6	>1.30
TDNA%	Spontaneous	1200	1000	22.3 ± 13	13.5 ± 8	0.82
	Initial	1200	999	41.1 ± 13.8	35.5 ± 11.7	0.44
	Residual	1199	1000	61.4 ± 16.8	28.1 ± 8.8	>1.30
OTM	Spontaneous	1200	1000	3.5 ± 2.3	1.9 ± 0.9	0.92
	Initial	1199	1000	12.7 ± 5.9	7.1 ± 3	>1.30
	Residual	1200	1000	20.4 ± 8.4	5.2 ± 2.4	>1.30

Effect size (ES): 0.2 low, 0.5 moderate, 0.8 high, 1.3 very high.
DNA: deoxyribonucleic acid.
TL: tail length, TDNA%: the percentage of DNA in the tail, OTM: olive tail moment.

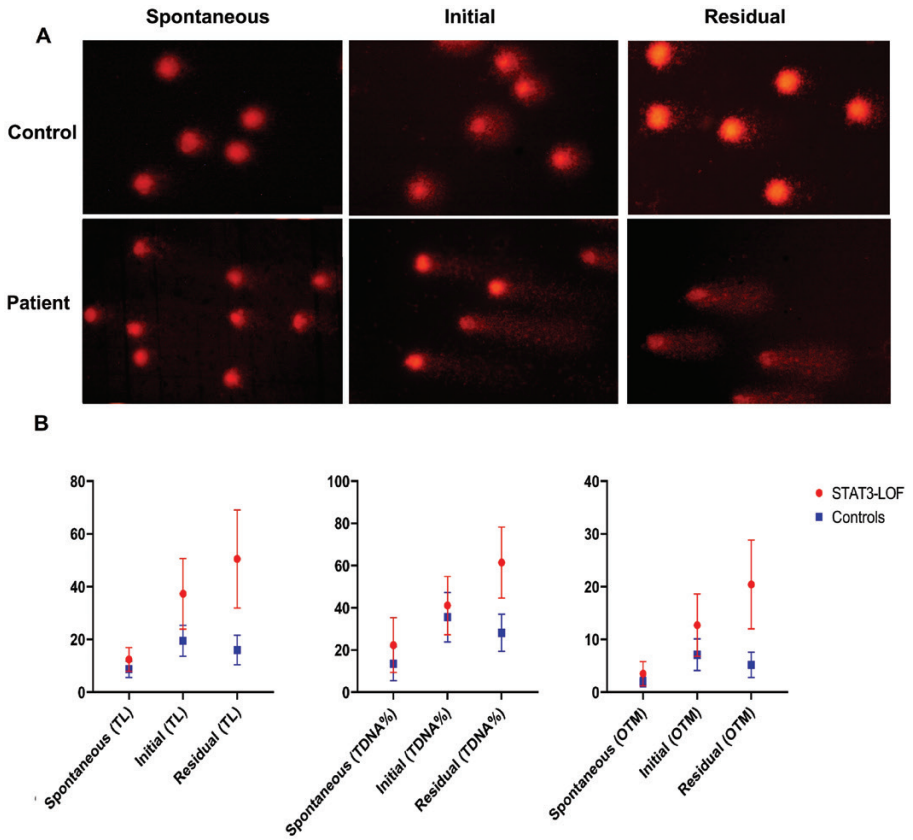

Figure 1: Radiation-induced DNA damage in leukocytes of patients with STAT3-LOF and controls. (A) Representative fluorescent microscope images of DNA comets and (B) boxplots of the evaluated comet parameters (tail length, tail % DNA, and olive tail moment).

It was determined that TL, TDNA%, and OTM parameters of patients with ZNF341 deficiency were higher in all measurements than controls; moreover, the effect size of this difference was substantially high. (Table 2, Figure 2).

No pathogenic variants were found in all exons of hereditary cancer susceptibility genes (MUTYH, NBN, PALB2, PIK3CA, PMS2, PMS2CL, PTEN, CHEK2, EPCAM, FAM175A, MLH1, MRE11A, MSH2, MSH6, TM, APC, BARD1, BRCA1, BRCA2, BRIP1, CDH1) and in all exons of cancer susceptibility genes (CDKN2A, FANCL, XRCC2, FANCA, FANCC, FANCB, FANCE, MLH1, PALB2, FANCG, FANCF, RAD50, APC, ERCC3, ERCC4, WT1, ERCC1, ERCC2, ATM, ERCC5, NOP10, BMPR1A) in patients with ZNF341 deficiency who developed malignancy.

It was observed that DNA scattering decreased in the parents' leukocytes 2 h after irradiation, unlike their ZNF341-deficient children.

Table 2 : Comet assay results of patients with ZNF341 deficiencies (n = 4) and controls (n = 4)

		Number of tested cells		Mean ± standard deviation		ES
		Patients	Controls	Patients	Controls	
TL	Spontaneous	400	400	11.78 ± 2.06	7.49 ± 2.49	>1.30
	Initial	400	400	36.73 ± 5.53	19.37 ± 3.78	>1.30
	Residual	400	400	53.59 ± 6.00	13.81 ± 3.52	>1.30
TDNA%	Spontaneous	400	400	20.43 ± 2.00	10.19 ± 2.01	>1.30
	Initial	400	400	41.42 ± 5.23	31.53 ± 3.56	>1.30
	Residual	400	400	58.38 ± 6.78	26.05 ± 2.75	>1.30
OTM	Spontaneous	400	400	3.29 ± 0.51	1.48 ± 0.41	>1.30
	Initial	400	400	11.35 ± 1.70	6.82 ± 1.17	>1.30
	Residual	400	400	20.91 ± 3.67	4.04 ± 0.81	>1.30

Effect size (ES): 0.2 low, 0.5 moderate, 0.8 high, 1.3 very high.
DNA: deoxyribonucleic acid.
TL: tail length, TDNA%: the percentage of DNA in the tail, OTM: olive tail moment.

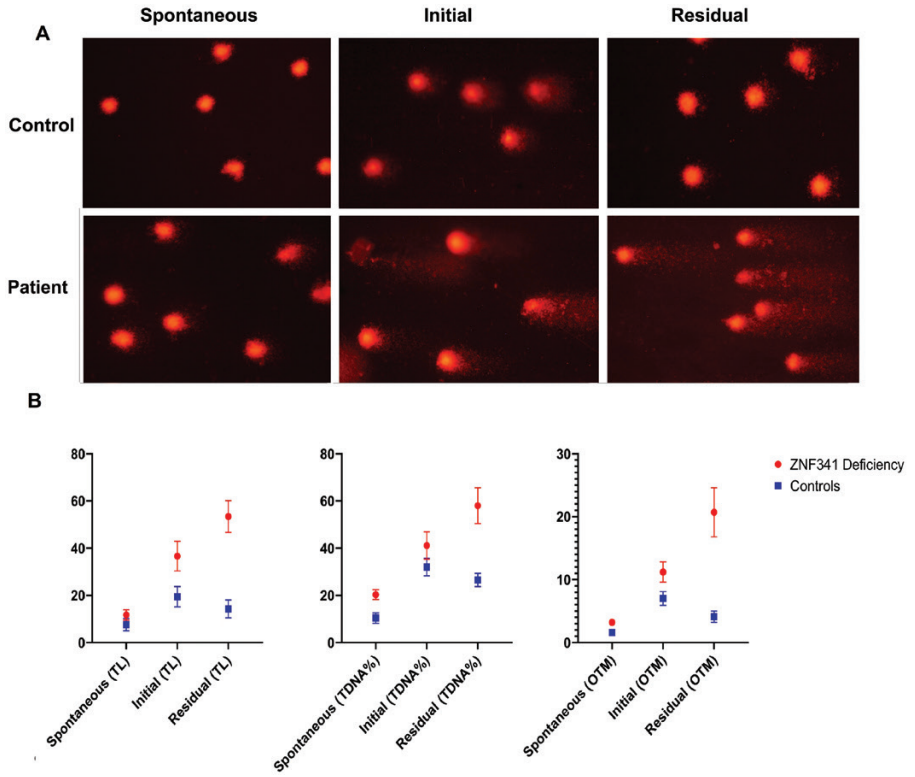

Figure 2: Radiation-induced DNA damage in leukocytes of patients with ZNF341 deficiency and controls. (A) Representative fluorescent microscope images of DNA comets and (B) boxplots of the evaluated comet parameters (tail length, percentage of DNA in the tail, and olive tail moment).

Ataxia telangiectasia

The TL, TDNA%, and OTM values at all stages of the comet test were higher in AT patients than in controls (Table 3).

Discussion

This study demonstrates for the first time that patients with STAT3-LOF and ZNF341 deficiency had increased radiation sensitivity and DNA repair defects compared with healthy controls. Our results highlight the need for close monitoring of these patients for the development of malignancy.

Circulating white blood cells can be used to reflect the overall body exposures to carcinogens and DNA repair activity in target tissues even if they are not the primary target cancer cells [16–19]. Thus, peripheral blood leukocytes are commonly used as surrogates to predict radiosensitivity based on DNA repair ability in healthy subjects and patients with or at risk of cancer. In this study, peripheral blood leucocytes of patients and controls were irradiated in the Comet test and the changes were examined.

STAT3 is a member of the STAT family that mediates signal transduction from the plasma membrane to the nucleus and

Table 3: Comet assay results of patients with Ataxia Telangiectasia (n = 3) and controls (n = 2)

		Number of tested cells		Mean ± standard deviation		ES
		Patients	Controls	Patients	Controls	
TL	Spontaneous	300	200	15.8 ± 4.7	7.2 ± 1.7	>1.30
	Initial	300	200	33.7 ± 9.1	15.2 ± 4.7	>1.30
	Residual	300	200	49.1 ± 12.3	13.5 ± 5.05	>1.30
TDNA%	Spontaneous	300	200	28.2 ± 8	10.1 ± 6.1	>1.30
	Initial	300	200	43.4 ± 13.2	27.8 ± 8.7	>1.30
	Residual	300	200	69 ± 16.5	19.9 ± 6.45	>1.30
OTM	Spontaneous	300	200	5.1 ± 2.1	1.65 ± 0.8	>1.30
	Initial	300	200	14.1 ± 5.1	4.5 ± 1.7	>1.30
	Residual	300	200	22.1 ± 6.5	3.2 ± 1.4	>1.30

Effect size (ES): 0.2 low, 0.5 moderate, 0.8 high, 1.3 very high.
DNA: deoxyribonucleic acid.
TL: tail length, TDNA%: the percentage of DNA in the tail, OTM: olive tail moment.

plays a role in various cellular activities [20]. It plays an important role in various complex and sometimes contradictory pathways such as proliferation, differentiation, migration, inflammation, and apoptosis [2]. The normal function of endogenous STAT3 is critical for reprogramming human somatic cells [21]. Although dysregulated STAT3 is known as a potent oncogene for many human cancers, it can act as both a potent tumor promoter and tumor suppressor factor [5, 22, 23]. It is well known that somatic activating gain-of-function (GOF) mutations in the STAT3 gene are associated with different hematological malignancies [24, 25]. Despite this association between somatic STAT3 GOF mutations and malignancy, cancer has been reported only in two patients [26]. It has been shown that hematological malignancies can also develop in patients with STAT3-LOF-mutations [27]. It was reported in the French National Survey that four (7%) of 60 STAT3-LOF patients developed non-Hodgkin lymphoma [28]. In another study involving 12 patients with STAT3-LOF, a case (8.3%) developed B-cell lymphoma [29]. Moreover, a case report shows the development of diffuse large B-cell lymphoma in a patient diagnosed with STAT3-LOF [30]. In our study, no malignancy has developed in our cases diagnosed with STAT3-LOF so far.

Ionizing radiation damages DNA directly and indirectly by increasing free radical species, especially OH radicals [31, 32]. STAT3 contributes to radioresistance in many cancer types by various mechanisms [31]. Therefore, STAT3 has become a promising target for the radiosensitization of cancer [31]. Pharmacological inhibition of STAT3 has increased radiosensitivity in various tumor types [33–35]. Additionally, STAT3 has a cytoprotective effect against oxidative stress and is required to efficiently repair damaged DNA [36, 37]. This is the first study to investigate radiation sensitivity in patients diagnosed with STAT3-LOF, the natural model of STAT3 deficiency, and our results showed that these patients had increased radiation sensitivity and insufficient DNA repair.

Human ZNF341 is essential for the STAT3 transcription-dependent auto-induction and sustained activity of STAT3, which appears to be necessary for the efficient repair of DNA damage [2]. The STAT3 error caused by the ZNF341 deficiency can facilitate cancer development by causing reduced resistance to radiation and DNA repair defects. In a recently published study, low expression levels of ZNF341 were associated with increased radiosensitivity in breast cancer [38]. In the Comet assay, as with STAT3 deficiency, patients with ZNF341 deficiency were found to have impaired DNA repair after radiation.

The number of patients diagnosed with ZNF341 is very low all over the world. Currently, 18 cases with ZNF341 deficiency have been reported in the literature, and cancer was detected in only one of them [8, 9]. As a risk factor, this patient had repeating X-ray imaging to demonstrate recurrent pulmonary infections and sinusitis. No documented chronic viremia or familial predisposition to cancer was found. In this patient, the absence of pathogenic variants in all exons of hereditary cancer susceptibility genes strengthens the possibility that malignancy (especially secondary cancer) development is associated with underlying genetic defect (ZNF341 deficiency) and radiation exposure.

Parents of patients with ZNF341 deficiency were also included in the study to understand whether post-radiation changes in the Comet test were due to a possible familial cancer tendency condition. However, DNA scattering tended to decrease at the second hour after irradiation, unlike their ZNF341-deficient children in parents. Therefore, this experiment supports the idea that there was no other familial pathology that led to radiosensitivity and impaired DNA repair.

The comet test has previously been shown to be a valid technique for measuring DNA damage in patients with AT [39]. It was also abnormal in the leukocytes of patients with AT, who were studied as positive controls in our study.

Our study has some limitations. ZNF341 deficiency and STAT3-LOF are rare inborn errors of immunity; as a result, we conducted it with a small number of patients. We think that longer follow-up is required to observe whether malignancy develops in patients. The development of malignancy occurs as a result of various mechanisms and complex events in primary immunodeficiencies. Although radiation sensitivity and disruption of DNA repair after irradiation are some of these mechanisms, they may not be sufficient alone to explain the development of cancer in these patients.

In conclusion, our study is the first to show radiation sensitivity with the comet test in cases with STAT3-LOF and

ZNF341 deficiencies. The data obtained from the Comet assay revealed a high level of radiation-induced DNA damage and deficiency in the repair process in all analyzed patient cells. The susceptibility to malignancy in these patients may be due to defects in DNA repair mechanisms. Closely monitoring patients with STAT3-LOF and ZNF341 deficiency for malignancies and protecting them from radiation exposure as much as possible would provide longer life expectancy and better outcomes. Further studies are needed to fully explain the pathogenesis of cancer development in these patients and to disclose whether the Comet test will be predictive on this issue.

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Conflict of interests

All authors declare that they have no competing interests.

Author contributions

S.C., T.C., and S.S.K. contributed to the study conception and design. Material preparation, data collection, and analysis were performed by S.C., H.H., M.H., N.K., G.O., A.O., E.K.A., A.M., C.D.A., S.G.T., S.O.S., S.B., T.C., and S.S.K. The first draft of the manuscript was written by S.C. and S.S.K., and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethical approval

Ethical approval was obtained from the Uludag University Faculty of Medicine Medical Research Ethics Committee for the study.

Patient consent

The informed consent form was obtained from participants.

Data availability

The data that support the study findings are available from the corresponding author upon reasonable request.

References

1. Mogensen TH. IRF and STAT transcription factors from basic biology to roles in infection, protective immunity, and primary immunodeficiencies. *Front Immunol* 2018, 9, 1–13. doi:10.3389/fimmu.2018.03047.
2. Vogel TP, Milner JD, Cooper MA. The Ying and Yang of STAT3 in human disease. *J Clin Immunol* 2015, 35, 615–23. doi:10.1007/s10875-015-0187-8.
3. Zhang Q, Boisson B, Beziat V, Puel A, Casanova JL. Human hyper-IgE syndrome: singular or plural?. *Mamm Genome* 2018, 29, 603–17. doi:10.1007/s00335-018-9767-2.
4. Avery DT, Deenick EK, Ma CS, Suryani S, Simpson N, Chew GY, et al. B cell-intrinsic signaling through IL-21 receptor and STAT3 is required for establishing long-lived antibody responses in humans. *J Exp Med* 2010, 207, 155–71. doi:10.1084/jem.20091706.
5. Hardee J, Ouyang Z, Zhang Y, Kundaje A, Lacroute P, Snyder M. STAT3 targets suggest mechanisms of aggressive tumorigenesis in diffuse large B-cell lymphoma. *G3 (Bethesda)* 2013, 3, 2173–85. doi:10.1534/g3.113.007674.
6. Barry SP, Townsend PA, Knight RA, Scarabelli TM, Latchman DS, Stephanou A. STAT3 modulates the DNA damage response pathway. *Int J Exp Pathol* 2010, 91, 506–14. doi:10.1111/j.1365-2613.2010.00734.x.
7. Laity JH, Lee BM, Wright PE. Zinc finger proteins: new insights into structural and functional diversity. *Curr Opin Struct Biol* 2001, 11, 39–46. doi:10.1016/s0959-440x(00)00167-6.
8. Béziat V, Li J, Lin JX, Ma CS, Li P, Bousfiha A, et al. A recessive form of hyper-IgE syndrome by disruption of ZNF341-dependent STAT3 transcription and activity. *Sci Immunol* 2018, 3, 1–40.
9. Frey-Jakobs S, Hartberger JM, Fliegauf M, Bossen C, Wehmeyer ML, Neubauer JC, et al. ZNF341 controls STAT3 expression and thereby immunocompetence. *Sci Immunol* 2018, 3, 1–25.
10. Cekic S, Hartberger JM, Frey-Jakobs S, Huriyet H, Hortoglu MB, Neubauer JC, et al. Cancer tendency in a patient with ZNF341 deficiency. *J Clin Immunol* 2020, 40, 534–8. doi:10.1007/s10875-020-00756-z.
11. Beedanagari S, Vulimiri SV, Bhatia S, Mahadevan B. Chapter 43 - Genotoxicity biomarkers: molecular basis of genetic variability and susceptibility, In: Gupta Ramesh C (ed), *Biomarkers in Toxicology*. Academic Press, Elsevier, 2014, 729–42.
12. Gunasekarana V, Raj GV, Chand P. A comprehensive review on clinical applications of comet assay. *J Clin Diagn Res* 2015, 9, GE01–5. doi:10.7860/JCDR/2015/12062.5622.
13. Schmeiser HH, Muehlbauer KR, Mier W, Baranski AC, Neels O, Dimitrakopoulou-Strauss A, et al. DNA damage in human whole blood caused by radiopharmaceuticals evaluated by the comet assay. *Mutagenesis* 2019, 34, 239–44.
14. Solange C, Teixeira JP. Comet Assay. In: Wexler Philip (ed), *Encyclopedia of Toxicology* (3rd edn). Academic Press, Elsevier, 2014, 1020–3.
15. McKenna DJ, McKeown SR, McKelvey-Martin VJ. Potential use of the comet assay in the clinical management of cancer. *Mutagenesis* 2008, 23, 183–90. doi:10.1093/mutage/gem054.
16. Buchynska LG, Brieieva OV, Iurchenko NP, Protsenko VV, Nespryadko SV. DNA damage in tumor cells and peripheral blood lymphocytes of endometrial cancer patients assessed by the comet assay. *Exp Oncol* 2017, 39, 299–303.
17. Najafzadeh M, Baumgartner A, Gopalan R, Davies JB, Wright A, Reynolds PD, Anderson D. In vitro sensitivities to UVA of lymphocytes from patients with colon and melanoma cancers and precancerous states in the micronucleus and the comet assays. *Mutagenesis* 2012, 27, 351–57.
18. Almeida GM, Duarte TL, Steward WP, Jones GD. Detection of oxaliplatin-induced DNA crosslinks in vitro and in cancer patients using the alkaline comet assay. *DNA Repair (Amst)* 2006, 5, 219–25. doi:10.1016/j.dnarep.2005.09.010.
19. Jianlin L, Jiliang H, Lifen J, Wei Z, Baohong W, Hongping D. Measuring the genetic damage in cancer patients during radiotherapy with three genetic end-points. *Mutagenesis* 2004, 19, 457–64. doi:10.1093/mutage/geh057.
20. Lim CP, Cao X. Structure, function, and regulation of STAT proteins. *Mol Biosyst* 2006, 2, 536–50.
21. Yu Z, Dmitrieva NI, Walts AD, Jin H, Liu Y, Ping X, et al. STAT3 modulates reprogramming efficiency of human somatic cells; insights from autosomal dominant Hyper IgE syndrome caused by STAT3 mutations. *Biol Open* 2020 Sep 10, 9, 1–11.
22. Tolomeo M, Cascio A. The multifaced role of STAT3 in cancer and its implication for anticancer therapy. *Int J Mol Sci* 2021, 22, 603, 1–25.
23. de la Iglesia N, Konopka G, Puram SV, Chan JA, Bachoo RM, You MJ, et al. Identification of a PTEN-regulated STAT3 brain tumor suppressor pathway. *Genes Dev* 2008, 22, 449–62.

24. Jerez A, Clemente MJ, Makishima H, Koskela H, Leblanc F, Peng Ng K, et al. STAT3 mutations unify the pathogenesis of chronic lymphoproliferative disorders of NK cells and T-cell large granular lymphocyte leukemia. *Blood* 2012, 120, 3048–57.
25. Koskela HL, Eldfors S, Ellonen P, van Adrichem AJ, Kuusanmäki H, Andersson EI, et al. Somatic STAT3 mutations in large granular lymphocytic leukemia. *N Engl J Med* 2012, 366, 1905–13.
26. Fabre A, Marchal S, Barlogis V, Mari B, Barbry P, Rohrlich PS, et al. Clinical aspects of STAT3 gain-of-function germline mutations: a systematic review. *J Allergy Clin Immunol Pract* 2019, 7, 1958–69.e9.
27. Yong PF, Freeman AF, Engelhardt KR, Holland S, Puck JM, Grimbacher B. An update on the hyper-IgE syndromes. *Arthritis Res Ther* 2012, 14, 228, 1–10.
28. Chandesris MO, Melki I, Natividad A, Puel A, Fieschi C, Yun L, et al. Autosomal dominant STAT3 deficiency and hyper-IgE syndrome: molecular, cellular, and clinical features from a French national survey. *Medicine (Baltimore)* 2012, 91, e1–e19.
29. Jiao H, Tóth B, Erdos M, Fransson I, Rákóczi E, Balogh I, et al. Novel and recurrent STAT3 mutations in hyper-IgE syndrome patients from different ethnic groups. *Mol Immunol* 2008, 46, 202–6.
30. Kumanovics A, Perkins SL, Gilbert H, Cessna MH, Augustine NH, Hill HR. Diff use large B cell lymphoma in hyper-IgE syndrome due to STAT3 mutation. *J Clin Immunol* 2010, 30, 886–93.
31. Wang X, Zhang X, Qiu C, Yang N. STAT3 Contributes to radioresistance in cancer. *Front Oncol* 2020, 10, 1120, 1–9.
32. Zhang X, Wang X, Xu R, et al. YM155 decreases radiation-induced invasion and reverses epithelial-mesenchymal transition by targeting STAT3 in glioblastoma. *J Transl Med* 2018, 16, 79, 1–11.
33. Pan Y, Zhou F, Zhang R, Claret FX. Stat3 inhibitor stattic exhibits potent antitumor activity and induces chemo- and radio-sensitivity in nasopharyngeal carcinoma. *PLoS One* 2013, 8, e54565, 1–3.
34. Adachi M, Cui C, Dodge CT, Bhayani MK, Lai SY. Targeting STAT3 inhibits growth and enhances radiosensitivity in head and neck squamous cell carcinoma. *Oral Oncol* 2012, 48, 1220–6.
35. Zhang Q, Zhang C, He J, Guo Q, Hu D, Yang X, et al. STAT3 inhibitor static enhances radiosensitivity in esophageal squamous cell carcinoma. *Tumour Biol* 2015, 36, 2135–42.
36. Barry SP, Townsend PA, McCormick J, Knight RA, Scarabelli TM, et al. STAT3 deletion sensitizes cells to oxidative stress. *Biochem Biophys Res Commun* 2009, 385, 324–9.
37. Barry SP, Townsend PA, Knight RA, Scarabelli TM, Latchman DS, Stephanou A. STAT3 modulates the DNA damage response pathway. *Int J Exp Pathol* 2010, 91, 506–14.
38. Yan D, Shen M, Du Z, Cao J, Tian Y, Zeng P, et al. Developing ZNF gene signatures predicting radiosensitivity of patients with breast cancer. *J Oncol* 2021, 2021, 9255494, 1–16.
39. Moreno Galarraga L, Santos Pérez JL, Ramirez-Tortosa MC, et al. Use of the comet test to assess DNA damage in children with ataxia-telangiectasia and their relatives. *J Investig Allergol Clin Immunol* 2008, 18, 137–8.