Am J Transl Res 2022;14(5):3180-3188
www.ajtr.org /ISSN:1943-8141/AJTR0137497

Original Article

Increased serum concentrations of estrogen-induced growth factors Midkine and FGF2 in NF1 patients with plexiform neurofibroma

Isis Atallah[1,2], Ana M Cieza Rivera[3], Octavio M Rivero Lezcano[3,4,5], Laura Tascón-González[4], Carolina González-Cortés[4], Cristina Diez Tascón[3], Tania Fernández-Villa[3], Vicente Martín[2,3,6]

[1]Division of Genetic Medicine, Lausanne University Hospital, 1011 Lausanne, Switzerland; [2]Faculty of Health Sciences, Department of Biomedical Sciences, Area of Preventive Medicine and Public Health, Universidad de León, León 24071, Spain; [3]Research Group in Gen-Environmental and Health Interactions (GIIGAS)-Institute of Biomedicine (IBIOMED), Universidad de León, León, Spain; [4]Unit of Investigation, Hospital of León, León, Spain; [5]Institute of Biomedical Research of Salamanca (IBSAL), Spain; [6]CIBER de Epidemiología y Salud Pública (CIBERESP), Madrid, Spain

Received July 19, 2021; Accepted December 4, 2021; Epub May 15, 2022; Published May 30, 2022

Abstract: Neurofibromatosis type 1 (NF1) predisposes to the development of dermal and plexiform neurofibromas and serum of NF1 patients stimulates neurofibroma proliferation in vitro. This study aimed to determine whether, in NF1 patients, serum levels of midkine (MK) and fibroblast growth factor 2 (FGF2) were associated with the number and/or type of neurofibromas. In addition, their concentrations were correlated with serum levels of dehydroepiandrosterone sulfate (DHEAS), a neurosteroid secreted by the peripheral nervous system. We performed a case control-study and measured, by ELISA assay, serum concentrations of MK, FGF2, and DHEAS in 20 NF1 patients and 30 controls. We found increased serum levels of MK and FGF2 in NF1 patients between 30 and 50 years old. Their concentrations were significantly higher in NF1 patients with plexiform neurofibromas than in controls (P=0.003 for MK and P=0.008 for FGF2). As an underlying hormonal regulation was suspected, DHEAS serum levels were measured but no difference was observed between patients and controls. We also observed a strong association between MK and FGF2 levels (P=0.0001) in NF1 patients and controls. In conclusion, we point out MK and FGF2 as biomarkers for plexiform neurofibroma in NF1 patients. As both growth factors are estrogen-responsive genes and neurofibromin is a co-repressor of estrogen receptor alpha activity, we suggest that the increased serum levels of MK and FGF2 observed in NF1 patients might be due to estradiol hypersensitivity.

Keywords: Neurofibromatosis type 1, midkine (MK), fibroblast growth factor 2 (FGF2), neurofibroma, plexiform, dehydroepiandrosterone sulfate (DHEAS), ELISA

Introduction

Neurofibromatosis type 1 (NF1, OMIM #162200) is caused by monoallelic variants in the tumor suppressor gene *NF1* [1]. NF1 affects one in 3,000 individuals worldwide [1]. Although the cardinal feature of NF1 is the development of benign peripheral nerve sheath tumors such as cutaneous (cNFs) and plexiform neurofibromas (pNFs), NF1 is also characterized by the presence of multiple café-au-lait spots, inguinal or axillary freckling, Lisch nodules, bony dysplasia and optic pathway gliomas [2]. cNFs are fleshy nodules in the skin which usually grow during adolescence and progress over the entire life span [3]. They might cause itching, pain and an important cosmetic burden with detrimental psychological effects [4]. Extreme variability in the number and size of cNFs is observed among affected individuals, even within the same family members, ranging from a few to several hundred [5]. pNFs occur in 20-50% of all patients with NF1 [6]. They are usually congenital tumors that appear at birth as a hyperpigmented macule with hypertrichosis [7] and might spread through a nerve plexus causing significant disfigurement, pain and neurological deficits [8]. The growth rate is variable during the lifetime. Thus, about 30% of pNFs will undergo malignant transformation into

malignant peripheral nerve sheath tumors (MPNST) [9], which have a poor prognosis [10]. The esthetic impact of cNFs and pNFs has been described as the most distressing symptom for NF1 patients [11].

Serum from NF1 patients enhanced proliferation of human neurofibroma-derived primary Schwann cells and endothelial cells "in vitro" [12], suggesting that it might contain some growth factors that stimulate the number and growth of neurofibromas. Previous studies have shown expression of MK and FGF2 in S-100 positive Schwann cells of the skin and blood vessels of cNFs, pNFs and MPNSTs of NF1 patients [13, 14]. MK is a heparin-binding growth factor, intensely expressed in the mid-gestation involved in neurogenesis, epithelial-mesenchymal interactions and mesoderm remodeling during embryogenesis [15, 16]. Thus it plays a critical role in growth, survival, migration, angiogenesis and cellular carcinogenesis [17]. MK expression is enhanced in many human carcinomas such as lung, gastrointestinal, urinary bladder and prostate carcinoma [18, 19]. FGF2 is a potent mitogen expressed widely during embryogenesis [20], proliferation, differentiation, survival, motility [21] and angiogenesis [22]. Increased FGF2 levels have been observed in plasma samples of patients affected by diverse malignancies, such as leukemia and lung and breast cancers, especially when metastases are present [23, 24].

Despite several studies, no biomarker has been found to predict the clinical burden of neurofibromas in NF1 patients. Identifying the growth factors involved in benign peripheral nerve sheath tumor growth might allow researchers to develop treatment strategies. This study aimed to investigate whether serum levels of MK and FGF2 were correlated with the number and/or type of neurofibromas in NF1 patients. In addition, as an underlaying hormonal regulation was suspected, their levels were correlated with serum levels of dehydroepiandrosterone sulfate (DHEAS), a neurosteroid secreted by the peripheral nervous system.

Materials and methods

Study design

This study was designed as a nested case-control study. Cases were identified by using the database of the Public Health Primary Care system and the database from the Complejo Asistencial Universitario de Leon. Only patients that fulfilled the National Institute of Health diagnosis criteria for NF1 (13) were included. Patients with optic glioma, malignant peripheral nerve sheath tumors or other kinds of malignancy were excluded to avoid confounding factors. Twenty patients provided written consent and underwent clinical examination. For each case, 1 to 2 controls from the León Health Area were included and matched for sex and age. The Institutional Review Board and Ethics Committee approved the study protocol (approval number 1060).

Patients

The study sample consisted of 20 cases (mean age 40.2 years, SD 16.8 years; 50% women) and 30 controls (mean age 41.4 years, SD 17.4 years; 50% women). Nine patients carried a plexiform neurofibroma. cNFs were counted and scored as following: 0 cNF, 1-50 cNFs and >50 cNFs. Clinical features of NF1 patients are shown in **Table 1**. For statistical analysis, the age was recoded into three categories (≤30 years, 30-50 years and >50 years).

Serum collection and enzyme-linked immunosorbent assay

Blood samples were collected from NF1 patients and controls, stored at room temperature for 30 min and subsequently centrifuged at 1250 × g for 13 minutes at 4°C (Eppendorf Centrifuge 5810R). The supernatant was transferred into microtubes and frozen at -80°C until use. MK and FGF2 concentrations were measured by enzyme-linked immunosorbent ELISA assay according to manufacturer's protocol: MK and FGF2 ELISA kits from Antigenix America and DHEAS Elisa Kit from Elabscience. ELISA range detection for MK: 23.44-3000 pg/ml, FGF2 range: 23.44-3000 pg/ml and DHEAS range: 15.63-1000 ng/ml.

Statistical analysis

Descriptive statistics were used to describe patient baseline characteristics. Quantitative variables were assessed with measures of centralisation and dispersion, while qualitative variables were described by relative frequencies with 95% confidence intervals. The correlation between MK and FGF2 levels was assessed

Table 1. MK, FGF2 and DHEAS serum levels and clinical features of NF1 patients

Case	Sex/ age	cNF	pNF	Location	CAL	Frecking	Optic glioma	Lisch nodules	Osseous dysplasia	Inheritance	MK (ng/ml)	FGF2 (ng/ml)	DHEAS (ng/ml)
1	F/9*	0	1	Sacral plexus	6-20	Diffuse	No	No	Severe scoliosis	Maternal	0.31	1.06	138.9
2	M/21**	0	0		6-20	Axillary	No	No, choroidal nevus	No	Paternal	0.62	1.73	118.3
3	M/22	1-50	1	Sacral plexus	6-20	Axillary	No	No	Sphenoidal dysplasia	“De novo”	ND	0.97	191.2
4	F/22	1 to 50	1	Back	6-20	Axillary, submammary	No	Yes	No	“De novo”	0.01	ND	140.5
5	F/22	1-50	1	Sacral plexus	6-20	Diffuse	No	No	No	“De novo”	ND	1.94	167.1
6	M/28	0	1	Sacral plexus	6-20	Diffuse	No	Yes	Left leg hypertrophy	“De novo”	0.54	1.94	242.0
7	F/32	1-50	0		6-20	Yes	No	No	No	“De novo”	ND	1.97	114.1
8	F/35	1-50	1	Abdominal	6-20	Axillary	No	No	No	Paternal	2.26	25.4	162.3
9	F/42	>50	0		6-20	No	No	No	No	“De novo”	1.34	17.18	130.2
10	M/42	1-50	1	Ankle	6-20	Axillary	Yes	Yes	Tibial dysplasia Severe scoliosis	“De novo”	1.55	12.72	134.1
11	M/43	1-50	1	Right arm	6-20	Axillary	No	No data	Severe scoliosis	“De novo”	0.82	6.46	142.5
12	F/44	1-50	0		6-20	Diffuse	Yes	No	Severe scoliosis	“De novo”	ND	0,27	172.8
13	F/48	>50	0		>20	Diffuse	No	Yes	No	Paternal	0.24	1.52	134.7
14	M/51	0	0		6-20	Diffuse	No	No	Kyphoscoliosis	“De novo”	0.19	ND	210.8
15	F/53*	>50	0		6-20	Axillary, inguinal, submammary	No	Yes + Iris mammillations	No	Maternal	1.10	8.42	321.9
16	M/54**	>50	1	Paratracheal	6-20	Axillary	No	No	No	“De novo”	0.14	ND	158.8
17	M/60	1-50	0		6-20	No	No	Yes	No	Maternal	0.07	ND	141.9
18	M/61	1-50	0		6-20	Axillary	No	Yes	No	Paternal	0.91	5.80	207.6
19	F/63*	>50	0		<6	Axillary	No	Yes	Partial sphenoid agenesis	Maternal	0.94	6.27	440.7
20	M/76	1-50	0		6-20	Axillary	No	Yes	No	Paternal	0.14	ND	164.2

Abbreviations: NF1: neurofibromatosis type 1; cNF: cutaneous neurofibroma; pNF: plexiform neurofibroma; CAL: café-au-lait macules; MK: midkine; FGF2: fibroblast growth factor 2; DHEAS: dehydroepiandrosterone sulfate; ND: not detected. * and ** are 2 members belonging to two different NF1 families.

by linear regression. Student's t-test and ANOVA test were performed to compare means.

Levene's test was performed to check homogeneity of variance. The Mann-Whitney test was used to compare the non-parametric values between two groups and the Kruskal-Wallis test was used to check the heterogeneity of the samples and to compare the non-parametric values between n groups. A significance level of 5% was considered in all analyses. The Statistical Package for Social Sciences (SPSS®) for Mac (Version 26) (IBM) was used for the statistical analysis.

Results

MK levels

The mean serum MK concentration was 0.56 ng/ml (SD 0.63 ng/ml) in NF1 patients and 0.40 ng/ml (SD 0.30 ng/ml) in healthy subjects (P=0.913). No difference in gender, number of cNF or presence of pNF was observed. MK serum levels showed an age dependent pattern (P=0.083) with 0.25 ng/ml in <30 years group, 0.89 ng/ml in the 30-50 years group, and 0.50 ng/ml in patients older than 50 years (**Figure 1**). In controls, serum MK concentrations varied slightly with age: 0.36 ng/ml, 0.46 ng/ml and 0.41 ng/ml for <30 years, 30-50 years and >50 years, respectively (P=0.524) (**Figure 1**). Higher serum MK levels were found in NF1 patients than in controls aged between 30 to 50 years, but the difference was not significant (P=0.245). However, in this age group, NF1 patients with a pNF had 3 fold levels of MK compared with NF1 patients without pNF (1.54 ng/ml versus 0.44 ng/mL; P=0.076) (**Figure 1**). When comparing with controls, MK levels were significantly increased in NF1 patients carrying pNF (0.71 ng/ml versus 3.32 ng/ml respectively; P=0.003).

FGF2 levels

The mean concentration of serum FGF2 for NF1 patients was 4.66 ng/ml (SD 6.79 ng/ml) and 2.65 ng/ml (SD 4.02 ng/ml) for controls (P=0.390). FGF2 levels showed a triphasic age-dependent pattern in NF1 patients (P=0.056) (1.27 ng/l, 9.28 ng/L and 2.93 ng/l). FGF2 serum levels in controls declined with age: 3.24 ng/l, 2.91 ng/l and 1.295 ng/l for <30 years, 30-50 years and >50 years groups (P=0.161) (**Figure 1**). Analysis of FGF2 concentration in NF1 patients did not reveal any association with gender, number of cNF or presence of a pNF. Despite higher levels of FGF2 in NF1 patients than in controls in the age group 30 to 50 years, difference was not significant (P=0.112; **Figure 1**). In this age group, NF1 patients with pNF showed higher FGF2 levels in comparison with NF1 patients without pNF (14.935 ng/l versus 4.744 ng/l; P=0.198) (**Figure 1**). This difference reached significance when compared with controls (6.12 ng/l in controls; P=0.006).

Relationship between serum MK and FGF2 concentrations

A strong relationship was observed between MK and FGF2 expression levels in both NF1 patients and controls (P≤0.0001; **Figure 2**).

DHEAS levels

The mean serum DHEAS concentration was 181 ng/ml (SD 78 ng/ml) in NF1 patients and 159 ng/ml (SD 35.8 ng/ml) in healthy subjects (P=0.235) (**Figure 1**). No differences in gender, number of cNF and presence of pNF (**Figure 1**) were observed.

Discussion

Several studies have previously attempted to identify the growth factors involved in neurofibroma proliferation in the serum of NF1 patients. Higher serum concentrations of fetal antigen-1 [25], melanocyte inhibitory activity and melanin related metabolite 5-S-cysteinyldopa [26], MK and Stem cell factor [27] have been previously found in NF1 patients, although their levels were not associated with type or number or neurofibromas.

In this study, we found that NF1 patients carrying a pNF have higher serum levels of MK and FGF2. However, this difference was observed only in the cohort of patients aged between 30 and 50 years. While an increase in serum MK levels upon reaching adulthood has been observed in a prior study [27], a decline of MK levels with older age in NF1 patients has not been reported so far; the fact that it showed up in our study may be related to the higher mean age of our NF1 patients. An age-related pattern of FGF2 serum levels in humans has not been

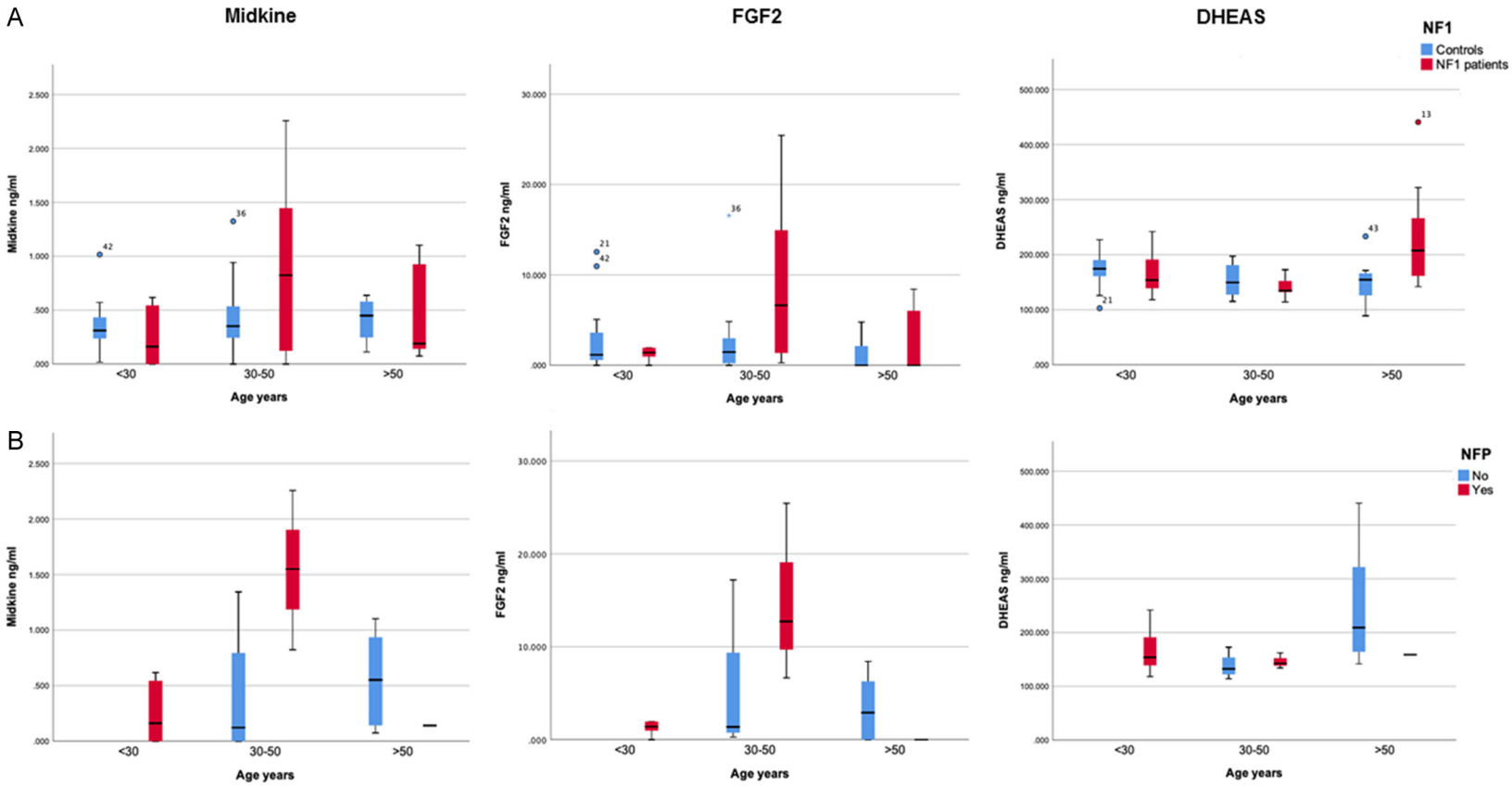

Figure 1. Mean MK, FGF2 and DHEAS serum levels. (A) According to NF1 status among the different age groups (<30, 30-50 and >50 years) and (B) according to the presence of pNF in NF1 patients among the different age groups (<30, 30-50 and >50 years).

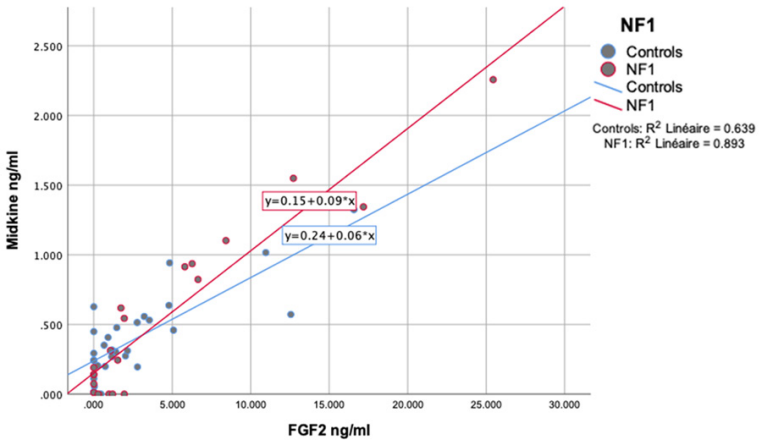

Figure 2. Strong association of MK and FGF2 serum levels in NF1 patients and controls.

described yet but a down-regulation of FGF2 has been observed in senescent mouse embryonic fibroblasts [28] and human mesenchyme-derived progenitor cells [29].

Unlike previously reported [27], we did not find a significant difference in MK levels between NF1 patients and controls. This might be due to our small number of patients, which is the main limitation of this study, and most importantly to our exclusion criteria. To avoid bias, we excluded NF1 patients presenting with malignancies that are known to express high MK levels (>1 ng/mL).

The age-related levels of both growth factors and the preferential growth of neurofibromas during puberty and pregnancy [30, 31] might suggest an underlying hormonal regulation. We considered dehydroepiandrosterone (DHEA) and its sulfate-ester (DHEAS) as hormone candidates to explain neurofibroma proliferation as they are among the neurosteroids secreted by the peripheral nervous system [32] and they show a similar age-dependent secretion pattern [33]. Serum DHEAS levels are high during fetal life, fall rapidly after birth and remain low until adrenarche. Serum levels increase again around age of 25-30 years followed by an age-dependent decline [34]. Although DHEAS was an interesting candidate, we did not observe any difference in DHEAS levels between NF1 patients and controls.

Surprisingly, we did find a strong association between the serum levels of the two distinct growth factors, MK and FGF2, both in NF1 patients and in controls. To our knowledge, this is the first time that such a relationship is observed between both growth factors. A similar strong linear correlation was observed for 17β-estradiol (E2) and MK in the serum of patients with lung adenocarcinoma [35]. In that study, E2 was able to enhance MK transcription by inducing recruitment of estrogen receptor β (ER-β) to the estrogen-responsive element (ERE) in the MK promoter in lung adenocarcinoma cells. They observed an analogous effect for ER-α in MCF-7 breast cancer cells [35]. FGF2 is also known to be an estrogen response gene [36, 37]. Thus, E2 induce FGF2 secretion mainly through the classical ER-α and -β in both normal and cancer cells [37-40], but also through the GPER/EGFR/ERK/c-fos/AP-1 signaling cascade in cancer-associated fibroblasts [41]. Noteworthy, DHEA and DHEAS are the main precursors of active estrogens in women [42] and DHEA is also able to stimulate GPER and ER-α36 [43].

This E2 signaling pathway might be relevant in NF1 patients as neurofibromin was shown to repress ERα signaling in a GTP independent manner, by localizing with ER on an ERE in breast cancer cells [44]. In that study, neurofibromin depletion causes estradiol hypersensitivity by increasing ER recruitment to EREs and expression of estrogen response genes [44]. Therefore, we could hypothesize that NF1 patients who carried abnormal neurofibromin might display an increased estradiol sensitivity leading to increased expression of FGF2 and MK (**Figure 3**).

In conclusion, our study identifies MK and FGF2 as biomarkers for pNF in NF1 patients. We observed an age-dependent secretion pattern of both growth factors as well as a strong association between serum levels of FGF2 and MK in NF1 patients and controls. As both growth factors are estrogen-responsive genes, we hypothesized that they might be simultaneously expressed in response to E2. FGF2 ER-like signaling could also contribute to MK secretion.

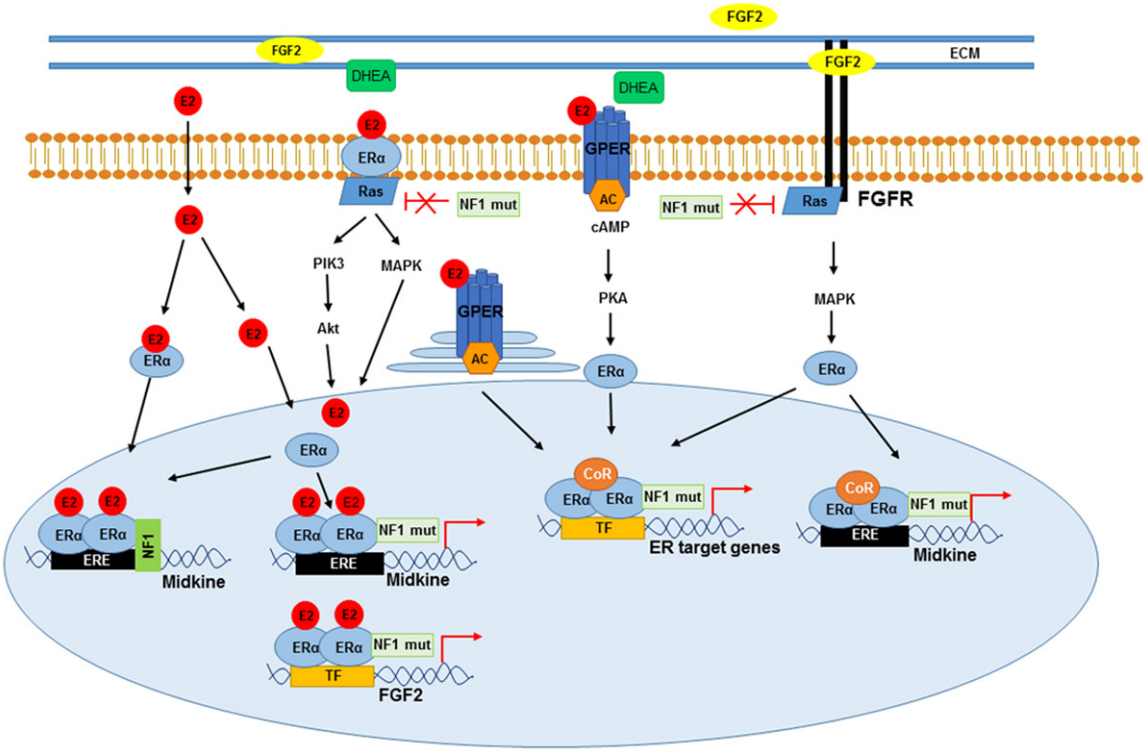

Figure 3. Illustration depicts the hypothetical relationship between midkine, FGF2, ER and neurofibromin in NF1 patients. E2 binds to ERα in the membrane, cytoplasm and/or nucleus. Activated ER forms homodimers recognize the ERE sequence and induce gene transcription [45]. Growth factors can also induce ER activity as a transcription factor and induce gene transcription. Neurofibromin acts as a co-repressor of E2 induced gene transcription [44]. In NF1 patients, mutant neurofibromin may lack E2 repressor activity and may allow transcription of genes that should be repressed, such as *MK* and *FGF2*. E2 can also bind GPER, which mediates non-genomic effects of estrogen and actives the G protein-dependent signaling pathway [46]. Thus GPER mediates a feedforward FGF2/FGFR1 paracrine activation [41]. DHEA is also able to induce estrogen signaling by binding to GPER and ER-α36 [43]. Receiver operating characteristic curve and diagnostic values or midkine as tumor marker for colorectal carcinoma. The area under the curve is 0.868 indicating that the diagnostic accuracy can be described as good. The optimal cut-off value of 56.42 pg/mL yields a balanced relationship between 84.3% sensitivity and 75.4% specificity. Abbreviations: CoR, coregulator; DHEA, dehydroepiandrostenedione; E217-beta, estradiol; ER, estrogen receptor; ERE, estrogen responsive element; FGF2, fibroblast growth factor 2; FGFR, fibroblast growth factor receptor, ECM, extracellular matrix, GPER, G protein-coupled estrogen receptor; MK, midkine; NF1, neurofibromin; mut, mutant.

Both mechanisms are relevant in NF1 patients as neurofibromin deficiency might lead to estradiol hypersensitivity and increased expression of estrogen-related genes. These hypotheses might need additional exploration.

Acknowledgements

We wish to express our gratitude to the patients who participated in this study and we thank Prof. Superti-Furga for his expertise and help in reviewing and revising this manuscript. This study was supported by a research grant from the Conserjería de Sanidad de la Junta de Castilla y León (GRS 571/A/10; 2010-12) assigned to Isis Atallah.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Isis Atallah, Division of Genetic Medicine, CHUV, Avenue Pierre Decker 5, CH 1011 Lausanne, Switzerland. Tel: +41-213140000 Ext. 61173; Fax: +41-213143392; E-mail: maria-isis.atallah-gonzalez@chuv.ch

References

[1] Gutmann DH, Aylsworth A, Carey JC, Korf B, Marks J, Pyeritz RE, Rubenstein A and Viskochil D. The diagnostic evaluation and multidisciplinary management of neurofibromatosis 1

and neurofibromatosis 2. JAMA 1997; 278: 51-57.
[2] Fibromatosis N. Conference statement. National Institutes of Health Consensus Development Conference. Arch Neurol 1988; 45: 575-578.
[3] Jett K and Friedman JM. Clinical and genetic aspects of neurofibromatosis 1. Genet Med 2010; 12: 1-11.
[4] Ablon J. Social stigma in neurofibromatosis type 1. In: Upadhyaya M, Cooper D, editors. Neurofibromatosis type 1: molecular and cellular biology. Berlin, Heidelberg: Springer; 2012. pp. 673-682.
[5] Ortonne N, Wolkenstein P, Blakeley JO, Korf B, Plotkin SR, Riccardi VM, Miller DC, Huson S, Peltonen J, Rosenberg A, Carroll SL, Verma SK, Mautner V, Upadhyaya M and Stemmer-Rachamimov A. Cutaneous neurofibromas: current clinical and pathologic issues. Neurology 2018; 91 Suppl 1: S5-S13.
[6] Korf BR. Plexiform neurofibromas. Am J Med Genet 1999; 89: 31-37.
[7] Huson SM, Harper PS and Compston DA. Von Recklinghausen neurofibromatosis. A clinical and population study in south-east Wales. Brain 1988; 111: 1355-1381.
[8] Hirbe AC and Gutmann DH. Neurofibromatosis type 1: a multidisciplinary approach to care. Lancet Neurol 2014; 13: 834-843.
[9] Ferner RE and O'Doherty MJ. Neurofibroma and schwannoma. Curr Opin Neurol 2002; 15: 679-684.
[10] Ducatman BS, Scheithauer BW, Piepgras DG, Reiman HM and Ilstrup DM. Malignant peripheral nerve sheath tumors. A clinicopathologic study of 120 cases. Cancer 1986; 57: 2006-2021.
[11] Wolkenstein P, Zeller J, Revuz J, Ecosse E and Leplege A. Quality-of-life impairment in neurofibromatosis type 1: a cross-sectional study of 128 cases. Arch Dermatol 2001; 137: 1421-1425.
[12] Mashour GA, Driever PH, Hartmann M, Drissel SN, Zhang T, Scharf B, Felderhoff-Müser U, Sakuma S, Friedrich RE, Martuza RL, Mautner VF and Kurtz A. Circulating growth factor levels are associated with tumorigenesis in neurofibromatosis type 1. Clin Cancer Res 2004; 10: 5677-5683.
[13] Mashour GA, Wang HL, Cabal-Manzano R, Wellstein A, Martuza RL and Kurtz A. Aberrant cutaneous expression of the angiogenic factor midkine is associated with neurofibromatosis type-1. J Invest Dermatol 1999; 113: 398-402.
[14] Mashour GA, Ratner N, Khan GA, Wang HL, Martuza RL and Kurtz A. The angiogenic factor midkine is aberrantly expressed in NF1-deficient Schwann cells and is a mitogen for neurofibroma-derived cells. Oncogene 2001; 20: 97-105.
[15] Kadomatsu K, Huang RP, Suganuma T, Murata F and Muramatsu T. A retinoic acid responsive gene MK found in the teratocarcinoma system is expressed in spatially and temporally controlled manner during mouse embryogenesis. J Cell Biol 1990; 110: 607-616.
[16] Mitsiadis TA, Salmivirta M, Muramatsu T, Muramatsu H, Rauvala H, Lehtonen E, Jalkanen M and Thesleff I. Expression of the heparin-binding cytokines, midkine (MK) and HB-GAM (pleiotrophin) is associated with epithelial-mesenchymal interactions during fetal development and organogenesis. Development 1995; 121: 37-51.
[17] Muramatsu T. Midkine and pleiotrophin: two related proteins involved in development, survival, inflammation and tumorigenesis. J Biochem 2002; 132: 359-371.
[18] Kerzerho J, Adotevi O, Castelli FA, Dosset M, Bernardeau K, Szely N, Lang F, Tartour E and Maillere B. The angiogenic growth factor and biomarker midkine is a tumor-shared antigen. J Immunol 2010; 185: 418-423.
[19] Wang QL, Wang H, Zhao SL, Huang YH and Hou YY. Over-expressed and truncated midkines promote proliferation of BGC823 cells in vitro and tumor growth in vivo. World J Gastroenterol 2008; 14: 1858-1865.
[20] Arany E and Hill DJ. Fibroblast growth factor-2 and fibroblast growth factor receptor-1 mRNA expression and peptide localization in placentae from normal and diabetic pregnancies. Placenta 1998; 19: 133-142.
[21] Basilico C and Moscatelli D. The FGF family of growth factors and oncogenes. Adv Cancer Res 1992; 59: 115-165.
[22] Dow JK and deVere White RW. Fibroblast growth factor 2: its structure and property, paracrine function, tumor angiogenesis, and prostate-related mitogenic and oncogenic functions. Urology 2000; 55: 800-806.
[23] Ruotsalainen T, Joensuu H, Mattson K and Salven P. High pretreatment serum concentration of basic fibroblast growth factor is a predictor of poor prognosis in small cell lung cancer. Cancer Epidemiol Biomarkers Prev 2002; 11: 1492-1495.
[24] Brown WS, Tan L, Smith A, Gray NS and Wendt MK. Covalent targeting of fibroblast growth factor receptor inhibits metastatic breast cancer. Mol Cancer Ther 2016; 15: 2096-2106.
[25] Jensen CH, Schroder HD, Teisner B, Laursen I, Brandrup F and Rasmussen HB. Fetal antigen 1, a member of the epidermal growth factor superfamily, in neurofibromas and serum from patients with neurofibromatosis type 1. Br J Dermatol 1999; 140: 1054-1059.

[26] Yoshida Y, Furumura M, Tahira M, Horie T and Yamamoto O. Serum biomarker in neurofibromatosis type 1. J Dermatol Sci 2012; 67: 155-158.
[27] Mashour GA, Driever PH, Hartmann M, Drissel SN, Zhang T, Scharf B, Felderhoff-Muser U, Sakuma S, Friedrich RE, Martuza RL, Mautner VF and Kurtz A. Circulating growth factor levels are associated with tumorigenesis in neurofibromatosis type 1. Clin Cancer Res 2004; 10: 5677-5683.
[28] Li J, Song S, Li X, Zhu J, Li W, Du B, Guo Y, Xi X and Han R. Down-regulation of fibroblast growth factor 2 (FGF2) contributes to the premature senescence of mouse embryonic fibroblast. Med Sci Monit 2020; 26: e920520.
[29] Hurley MM, Gronowicz G, Zhu L, Kuhn LT, Rodner C and Xiao L. Age-related changes in FGF-2, fibroblast growth factor receptors and beta-catenin expression in human mesenchyme-derived progenitor cells. J Cell Biochem 2016; 117: 721-729.
[30] Dugoff L and Sujansky E. Neurofibromatosis type 1 and pregnancy. Am J Med Genet 1996; 66: 7-10.
[31] Rosser T and Packer RJ. Neurofibromas in children with neurofibromatosis 1. J Child Neurol 2002; 17: 585-591.
[32] Baulieu EE. Neurosteroids: of the nervous system, by the nervous system, for the nervous system. Recent Prog Horm Res 1997; 52: 1-32.
[33] Orentreich N, Brind JL, Vogelman JH, Andres R and Baldwin H. Long-term longitudinal measurements of plasma dehydroepiandrosterone sulfate in normal men. J Clin Endocrinol Metab 1992; 75: 1002-1004.
[34] Rainey WE, Carr BR, Sasano H, Suzuki T and Mason JI. Dissecting human adrenal androgen production. Trends Endocrinol Metab 2002; 13: 234-239.
[35] Zhao G, Nie Y, Lv M, He L, Wang T and Hou Y. ERbeta-mediated estradiol enhances epithelial mesenchymal transition of lung adenocarcinoma through increasing transcription of midkine. Mol Endocrinol 2012; 26: 1304-1315.
[36] Siegfried JM, Gubish CT, Rothstein ME, Henry C and Stabile LP. Combining the multitargeted tyrosine kinase inhibitor vandetanib with the antiestrogen fulvestrant enhances its antitumor effect in non-small cell lung cancer. J Thorac Oncol 2012; 7: 485-495.
[37] Siegfried JM, Farooqui M, Rothenberger NJ, Dacic S and Stabile LP. Interaction between the estrogen receptor and fibroblast growth factor receptor pathways in non-small cell lung cancer. Oncotarget 2017; 8: 24063-24076.
[38] Huang C, Yuan P, Wu J and Huang J. Estrogen regulates excitatory amino acid carrier 1 (EAAC1) expression through sphingosine kinase 1 (SphK1) transacting FGFR-mediated ERK signaling in rat C6 astroglial cells. Neuroscience 2016; 319: 9-22.
[39] Fillmore CM, Gupta PB, Rudnick JA, Caballero S, Keller PJ, Lander ES and Kuperwasser C. Estrogen expands breast cancer stem-like cells through paracrine FGF/Tbx3 signaling. Proc Natl Acad Sci U S A 2010; 107: 21737-21742.
[40] Ren Y, Jia HH, Xu YQ, Zhou X, Zhao XH, Wang YF, Song X, Zhu ZY, Sun T, Dou Y, Tian WP, Zhao XL, Kang CS and Mei M. Paracrine and epigenetic control of CAF-induced metastasis: the role of HOTAIR stimulated by TGF-ss1 secretion. Mol Cancer 2018; 17: 5.
[41] Santolla MF, Vivacqua A, Lappano R, Rigiracciolo DC, Cirillo F, Galli GR, Talia M, Brunetti G, Miglietta AM, Belfiore A and Maggiolini M. GPER mediates a feedforward FGF2/FGFR1 paracrine activation coupling CAFs to cancer cells toward breast tumor progression. Cells 2019; 8: 223.
[42] Labrie F, Belanger A, Cusan L, Gomez JL and Candas B. Marked decline in serum concentrations of adrenal C19 sex steroid precursors and conjugated androgen metabolites during aging. J Clin Endocrinol Metab 1997; 82: 2396-2402.
[43] Teng Y, Radde BN, Litchfield LM, Ivanova MM, Prough RA, Clark BJ, Doll MA, Hein DW and Klinge CM. Dehydroepiandrosterone activation of g-protein-coupled estrogen receptor rapidly stimulates microRNA-21 transcription in human hepatocellular carcinoma cells. J Biol Chem 2015; 290: 15799-15811.
[44] Zheng ZY, Anurag M, Lei JT, Cao J, Singh P, Peng J, Kennedy H, Nguyen NC, Chen Y, Lavere P, Li J, Du XH, Cakar B, Song W, Kim BJ, Shi J, Seker S, Chan DW, Zhao GQ, Chen X, Banks KC, Lanman RB, Shafaee MN, Zhang XH, Vasaikar S, Zhang B, Hilsenbeck SG, Li W, Foulds CE, Ellis MJ and Chang EC. Neurofibromin is an estrogen receptor-alpha transcriptional co-repressor in breast cancer. Cancer Cell 2020; 37: 387-402, e387.
[45] Tecalco-Cruz AC and Ramirez-Jarquin JO. Mechanisms that increase stability of estrogen receptor alpha in breast cancer. Clin Breast Cancer 2017; 17: 1-10.
[46] Xu S, Yu S, Dong D and Lee LTO. G protein-coupled estrogen receptor: a potential therapeutic target in cancer. Front Endocrinol (Lausanne) 2019; 10: 725.