UNIVERSIDAD AUTONOMA DE GUERRERO

FACULTAD DE CIENCIAS QUÍMICO BIOLÓGICAS

DOCTORADO EN CIENCIAS BIOMÉDICAS

“Efecto de miR-24 sobre YKL-40 en leucemia linfoblástica aguda”

T E S I S

QUE PARA OBTENER EL GRADO DE

DOCTOR EN CIENCIAS BIOMEDICAS

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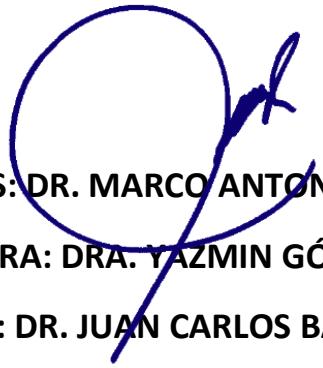

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CHILPANCINGO, GRO. **ENERO 2021**

El presente proyecto de investigación fue realizado en el Laboratorio de Biomedicina Molecular de la Facultad de Ciencias Químico Biológicas de la Universidad Autónoma de Guerrero.

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DEDICATORIA

Este trabajo académico que resume grandes aprendizajes, esfuerzos y metas cumplidas está dedicado a los motores de mi vida que me impulsan a salir adelante día con día mi hija Ana paula y mi esposo Rafael que me apoyo en todo momento durante esta etapa de mi vida. A mi Madre quien me hizo ser la mujer que hasta ahora soy y a mi Padre que siempre me ha impulsado a seguir adelante.

AGRADECIMIENTOS

A dios…

A mi **madre Natividad Alarcón Lobato** que aunque ya no está físicamente conmigo su esencia y la gran mujer que fue me han impulsado a luchar por mis sueños y cumplir mis metas.

A mi **esposo Rafael Encarnación Torres** quien me ha apoyado en todo momento, soportando mis malos humores y mis rachas de enojo, por tener siempre una palabra de aliento y no dejarme caer. Gracias. Te amo.

A mi adorable **hija Ana Paula** que es mi motor el cual me impulsa a seguir adelante día con día y me hizo más llevaderos los día con sus ocurrencias y travesuras.

A mi **hermana Yizel Rivas Alarcón** por ser mi confidente y gran amiga y por siempre aconsejarme y hacerme sentir orgullosa de mi misma y aprender siempre a confiar en mí.

A mi **padre Victor Manuel Rivas Saavedra** que con su ejemplo, palabras y consejos me ha impulsado a seguir adelante.

A mi **Tía Leticia Alarcón Lobato** un ángel en el cielo que me brindó su apoyo incondicional en todo momento cuando más la necesite estuvo siempre conmigo.

Al **doctor Marco Antonio Leyva Vázquez** por creer en mí y nunca dudar de que si podía, por sus consejos y asesorías, por sus bromas que siempre hacían pasar un rato agradable. Gracias doc.

A la **doctora Berenice Illades Aguiar** por confiar en mí, por abrirme las puertas de su laboratorio, por impulsarme a seguir adelante y seguir preparándome día con día.

Al doctor **Jorge Organista Nava** y a la doctora **Yazmin Gómez Gómez** por brindarme su amistad y su invaluable apoyo y consejos para la realización de este trabajo sin sus aportaciones no hubiera sido posible.

A mi **comité tutorial, Dra. Luz del Carmen Alarcón Romero, Dra. Natividad Castro Alarcón y Dr. Daniel Hernández Sotelo,** por todas sus aportaciones y críticas para que este trabajo se llevará a cabo de la mejor manera, por sus asesorías y por cada pregunta realizada para enriquecer esta investigación.

A la doctora **Eloisa Ibarra Sierra y** a la maestra **Monica Saavedra Herrera** por su incondicional apoyo en la realización de este proyecto en el área de investigación del Instituto estatal de Cancerología “Dr. Arturo Beltrán Ortega”.

A mi familia por estar siempre al pendiente de mí, por sus consejos y los buenos momentos que me hacían sentir más llevadero el camino.

A mis compañeros de laboratorio; la **química Natividad Sales Linares** por su amistad y consejos, a la **MC. Mireya Cisneros Villanueva** por su amistad y apoyo brindado al Dr. **Hugo Rodríguez Ruiz** y al **MC. Luis Antonio Martínez** por su apoyo y por las risas y ocurrencias. A todos los integrantes del laboratorio de Biomedicina Molecular que aportaron un granito de arena en la realización de esta investigación.

EL CAMINO NO FUE FÁCIL.. PERO LO LOGRE.

“Efecto de miR-24 sobre YKL-40 en leucemia linfoblástica aguda”

Efecto de miR-24 sobre YKL-40 en leucemia linfoblástica aguda

Í N D I C E **Pagina**

Resumen

Introducción En México el cáncer es un problema de salud pública, afectando a adultos y a población infantil. En el estado de Guerrero la leucemia linfoblástica aguda (LLA), es el tipo de cáncer más común que afecta a población menor de 18 años de edad. El pronóstico de un paciente con LLA, es difícil de establecer, se han planteado diversos factores que pueden influir en el pronóstico de un paciente con LLA, algunos de ellos son; la edad, alteraciones citogenéticas como las translocaciones, el sexo conteo inicial de leucocitos e invasión al sistema nervioso central (SNC). La búsqueda de nuevos biomarcadores pronósticos es importante y debe ser progresiva. Existen varios estudios que proponen a la proteína YKL-40 como biomarcador de supervivencia en diferentes tipos de cáncer, (pulmón, mama, hígado, vejiga y endometrio) ya que niveles elevados de esta proteína en suero o plasma se han relacionado con corta supervivencia y mal pronóstico. Se ha propuesto que miR-24 puede regular a YKL-40. Mir-24 es un microRNA asociado a la tumorigénesis y ha sido considerado un oncomiR, se ha estudiado en diferentes tipos de cáncer como en cáncer de mama, gástrico, páncreas y pulmón.

Metodología Se llevó a cabo un estudio de casos y controles. El grupo de casos los conformaron 45 niños que acudieron de nuevo ingreso al servicio de oncología pediátrica en el Instituto Estatal de Cancerología en Acapulco Guerrero, captados entre julio de 2016 a Septiembre de 2018. El diagnóstico de LLA fue confirmado mediante aspirado de medula ósea y fue subclasificado por inmunofenotipo. El grupo control lo conformaron 45 niños aparentemente sanos sin leucemia (4.5-11.0 x 10^3 leucocitos/mm^3) captados en el Centro de Salud “Dr. Ramón Carreto Leyva” en Chilpancingo Guerrero, México. Los participantes en ambos grupos de estudio fueron de 1 a 18 años de edad, incluyendo hombres y mujeres. Las muestras biológicas utilizadas en ambos grupos de estudio, fueron muestras de sangre periférica obtenidas a partir de punción venosa. Se separó el plasma de cada una de las muestras y se almaceno a -70°C. Se realizó la extracción y purificación de RNA utilizando trizol. Para evaluar los niveles de expresión de miR-24, se sintetizó cDNA a partir de RNA total, utilizando el kit de transcripción reversa Taqman MicroRNA, posteriormente se realizó una qPCR utilizando el ensayo Taqman MicroRNA. Para la cuantificación del RNAm de YKL-40, se sintetizó cDNA a partir de RNA total, posteriormente se llevó a cabo una qPCR utilizando el ensayo Taqman Probe spans exons. Los niveles en plasma de YKL-40 fueron medidos utilizando el kit de ELISA para YKL-40. Se llevó a cabo cultivo primario de linfocitos y cultivo de la linea celular Jurkat clon E6-1, posteriormente se realizó Western Blot para medir los niveles proteicos de YKL-40 en el cultivo primario de linfocitos y en la linea celular Jurkat clon E6-1.

Resultados La expresión de miR-24 y RNAm de YKL-40 son significativamente más altas en niños con LLA comparado con niños sin leucemia. Los niveles plasmáticos de YKL-40 son significativamente más altos en niños con LLA comparado con niños sin LLA, además niveles altos en plasma de YKL-40 se asociaron significativamente con una supervivencia más corta en niños con LLA. Nuestra investigación también reveló que los niveles en plasma de YKL-40, la edad, conteo inicial de leucocitos y la invasión al SNC, están asociados con el pronóstico de pacientes con LLA.

La expresión de miR-24 es mayor en el cultivo primario de linfocitos. No se detectó a YKL-40 en cultivo primario de linfocitos en comparación con células Jurkat y CaCu en donde si de detectaron niveles proteicos de YKL-40.

Conclusión Los resultados indican que miR-24 y el RNAm de YKL-40 se encuentran sobreexpresado en niños con LLA. No se observó regulación negativa de miR-24 sobre los niveles de expresión de YKL-40 en LLA. miR-24 y el RNAm de YKL-40 no se asocian con la supervivencia de niños con LLA. Niveles altos en plasma de YKL-40 se asocian con una supervivencia más corta en niños con LLA.

Palabras clave YKL-40, miR-24, supervivencia, leucemia linfoblástica aguda, pronóstico.

INTRODUCCION

En México el cáncer es un problema de salud pública, afectando a adultos y a población infantil, siendo la segunda causa de muerte en niños entre 5 y 14 años de edad. En el estado de Guerrero, entre 2008 y 2014 se presentaron 602 casos de cáncer en menores de 18 años de edad no derechohabientes, siendo la leucemia linfoblástica aguda (LLA), el tipo de cáncer más común en esta población con el 55.7% de los casos. (Rendón *et al.,* 2012, CENSIA, SSA/INEGI 2014, Rivera *et al.,* 2015).

El pronóstico de un paciente con LLA, es difícil de establecer, el comportamiento clínico puede ser parcialmente predicho por ciertas características que son consideradas factores pronósticos, entre ellas podemos mencionar la edad, alteraciones citogenéticas como las translocaciones, inmunofenotipo, sexo y conteo inicial de leucocitos. (Layton, 2015) (Carroll *et al*, 2012) (Jabbour *et al,* Kaspers *et al,* 2012). Debido a esto, la búsqueda de nuevos biomarcadores pronósticos es importante y debe ser progresiva.

Existen estudios que proponen que los miRNAs son una importante herramienta en la clasificación molecular de las leucemias y también se han estudiado como posibles biomarcadores en el pronóstico de la enfermedad. Los miRNAs podrían ser utilizados como un nuevo blanco molecular para el desarrollo de nuevas estrategias terapéuticas (Organista *et al.,* 2015, Fatica *et al.,* 2013, Fabbri *et al.,* 2008, Shuangli *et al.,* 2007, Chen *et al.,* 2004). Los microRNAs son pequeñas regiones de RNA no codificantes de cadena sencilla, de 20 a 22 nucleótidos que regulan la expresión de genes, ya sea por interferir en su traducción o en la estabilidad del RNAm. Los microRNAs comprenden del 1-3% del genoma y regulan la expresión del alrededor del 30% de genes humanos (Bentwich *et al.,* 2005). Una de las principales funciones de los microRNAs es el silenciamiento de genes por unirse a secuencias complementarias en la región 3´UTR de RNAs mensajeros blanco (McFarlane *et al.,* 2010, Gulyaeva *et al.,* 2016).

Varios estudios han demostrado que los microRNAs están asociados con multiples procesos fisiológicos celulares y en procesos biológicos esenciales como el desarrollo, hematopoyesis, envejecimiento y funciones endocrinas (Pasquinelli *et al.,* 2005, MacFarlane *et al.,* 2010).

miR-24 es uno de los microRNAs mejor conocido correlacionado con la tumorigénesis, es considerado un onocomiR. El gen que codifica para miR-24 se localiza en el brazo largo del cromosoma 9 región 22.32. miR-24 juega un papel importante en la regulación de la hematopoyesis, apoptosis y proliferación celular (Montagner *et al.,* 2014). Se ha encontrado sobreexpresión de miR-24 en una gran variedad de canceres humanos, incluyendo cáncer de mama (Cui *et al.,* 2017), cáncer pancreático (Siegel *et al.,* 2014), cáncer de pulmón de células no pequeñas (Zhao *et al.,* 2015), cáncer gástrico (Zhang *et al.,* 2016) y leucemias (Organista *et al.,* 2015). Datos publicados en un metaanálisis en el 2018 sugieren que la desregulación de miR-24 en cáncer, podría predecir una supervivencia global más corta. Con una asociación estadísticamente significativa (P=0.0002) (Quan *et al*., 2017).

miR-24 tiene varios blancos que participan en diferentes procesos, algunos de ellos son: Ciclina A2, CDK4, implicados en el ciclo celular, genes proapoptóticos como FAF-1, caspasa 9 y Apaf-1. (Lal *et al.,* 2009, Nguyen *et al.,* 2013). Estudios recientes han propuesto que miR-24 tiene como blanco a YKL-40 (Sun *et al.,* 2016).

La proteína tipo quitinasa YKL-40 es una proteína filogenéticamente conservada de unión a quitina y heparina, sin actividad quitinasa, es conocida también como glucoproteína 39 del cartílago humano y como proteína 1 tipo quitinasa 3 (chitinase-3-like protein 1). El nombre de proteína YKL-40, deriva de su peso molecular que es de 40 KDa y de un código de letras para los aminoácidos presentes en su extremo N-terminal, tirosina (Y), lisina (K) y leucina (L) (Kzhyshkowska *et al.,* 2007). En los seres humanos es codificada por el gen CHI3L1, localizado en el cromosoma 1 (1q31-q32) (Kzhyshkowska *et al.,* 2016) y consiste en diez exones de alrededor

de 8 Kb (Johansen *et al.,* 2006, Bergmmam *et al.,* 2005). La concentración media en suero de YKL-40 en adultos sanos es de 40 µg/L, no presentando diferencia entre géneros, en personas mayores los niveles de YKL-40 llegan a incrementar (Johansen *et al.,* 2006*).* La proteína YKL-40 se aisló por primera vez en cultivo de condrocitos articulares humanos y fibroblastos sinoviales (Kzhyshkowska *et al.,* 2007).

La proteína YKL-40 es secretada por diferentes tipos celulares, como células inflamatorias (neutrófilos y macrófagos), células madre (células embrionarias y fetales) células musculares vasculares lisas, condrocitos y por células cancerosas de diferentes orígenes (Prakash *et al.,* 2013, Schultz *et al.,* 2010). La función exacta de YKL-40 aún no es del todo clara, sin embargo hay estudios que revelan que juega un papel importante en la proliferación y diferenciación celular (Johansen *et al.,* 2007), angiogénesis (Shao *et al.,* 2009), inflamación (Kzhyshkowska et al., 2007), remodelamiento de la matriz extracelular (Recklies *et al.,* 2002) y apoptosis (Lee *et al.,* 2009).

La función biológica de YKL-40 en células cancerosas no es del todo clara, sin embargo hay estudios que indican que esta proteína puede tener diferentes papeles dependiendo del tipo celular y de su microambiente. Se ha sugerido que YKL-40 participa en la proliferación y el desarrollo de células malignas y las protege de sufrir apoptosis, estimula la angiogénesis y tiene un efecto en el remodelamiento extracelular del tejido, regulando la actividad de los fibroblastos e incrementando el grado de fibrosis alrededor de las células cancerosas en tumores sólidos, sin embargo no se han realizado estudios in vivo que lo demuestren (Schultz *et al.,* 2010)

Niveles elevados de YKL-40 en suero o plasma han sido detectados en pacientes con varios tipos de cáncer como cáncer de pulmón (Xu., *et* al 2014), de mama (Wang.,*et al* 2012), de endometrio (Kotowics.,*et al* 2017), colangiosarcoma (Thongsom .,*et al* 2016) y se han relacionado con corta supervivencia.

En un estudio llevado a cabo por Sun *et al.,* 2016 en líneas celulares de cáncer cervical, se concluyó que miR-24 tiene como blanco a YKL-40, sin embrago en otros tipos de cáncer como la leucemia linfoblástica aguda no se han llevado a cabo estudios que demuestren que miR-24 tiene efecto sobre YKL-40. Debido a ello en esta investigación se analizó si miR-24 tiene efecto sobre YKL-40 en leucemia linfoblástica aguda.

CAPITULO I

Plasma levels of YKL-40 is a prognostic factor in childhood acute lymphoblastic leukemia

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November 20, 2020

Our reference: 260568
Journal: Molecular and Clinical Oncology
Title: Plasma levels of YKL-40 is a prognostic factor in childhood acute lymphoblastic leukemia
Authors: Miss Alinne Ayulieth Rivas-Alarcón, Professor Yazmín Gómez-Gómez, Professor Jorge Organista-Nava, Dr Marco Antonio Jiménez-López, Dr Ana Bertha Rivera-Ramírez, Professor Eloisa Ibarra-Sierra, Miss Mónica Virginia Saavedra-Herrera, Professor Berenice Illades-Aguiar, Professor Marco Antonio Leyva-Vázquez

Dear Marco antonio Leyva vazquez,

Thank you for your submission. The manuscript 'Plasma levels of YKL-40 is a prognostic factor in childhood acute lymphoblastic leukemia' by Rivas-Alarcón et al was provisionally accepted for publication in Molecular and Clinical Oncology on November 20, 2020. The submission should be considered to be formally accepted upon the receipt of proofs.

An author PDF file will be provided on publication.

Yours Sincerely,

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Plasma levels of YKL-40 is a prognostic factor in childhood acute lymphoblastic leukemia

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Running tittle: RIVAS et al: YKL-40 PLASMA LEVELS IN PROGNOSIS OF PEDIATRIC ALL.

Abstract

YKL-40 also known as CHI3L1 (Chitinase-3-like protein 1), is an inflammatory glycoprotein which is secreted by different cell types such as inflammatory cells. This protein is elevated in the serum or plasma of patients with different types of cancer and high concentration of this protein is associated with poor prognosis and short survival in patients with liver, breast, lung, bladder, and endometrial cancers. In Mexico, acute lymphoblastic leukemia (ALL) is the most common type of cancer that affects the pediatric population. The prognosis of patients with ALL is difficult to establish. Hence, the objective of this study was to analyze the plasma levels of YKL-40 in Mexican children with ALL and investigated its role as a prognostic factor. We performed a case-control study in a population of 90 children aged 1 to 18 years, among whom 45 had ALL and 45 were hematologically healthy. YKL-40 levels in the subjects' plasma samples were measured using an ELISA kit. We found that the plasma levels of YKL-40 were significantly higher in children with ALL than in controls ($p<0.0001$). Children with ALL who had high plasma levels of YKL-40 (≥36.34 ng/mL) had shorter survival durations than those with low levels (<36.34 ng /mL) ($p<0.05$). Our investigation revealed that the YKL-40 plasma level, age/initial leukocyte count, and Central nervous system (CNS) invasion are associated with the prognosis of children with ALL (OR= 6.06, 95%IC = 1.1-31.6, p = 0.03; OR= 8.53, 95%IC= 1.2-58.2, p= 0.03 and OR= 6.45, 95%IC= 1.01-41.2, p= 0.04). YKL-40 plasma levels could therefore be used as a prognostic biomarker in children with ALL; however, more studies are required to confirm the role of this protein in patients with this disease.

Keywords: acute lymphoblastic leukemia, childhood, YKL-40, prognosis, survival.

Introduction

Childhood cancer is a public health challenge in Mexico, with more than 5,000 new cases diagnosed annually, and is the second leading cause of death in children between 5 and 14 years of age. In the Mexican state of Guerrero, acute lymphoblastic leukemia (ALL) is the most common malignancy in children (1-3). The prognosis of a patient with ALL can be partially predicted based on certain clinical characteristics such as age, karyotype, cytogenetic alterations, immunophenotype, sex, and initial leukocyte count (4, 5). Hence, it is important to identify novel and more reliable prognostic biomarkers for leukemia.YKL-40 protein, also known as CHI3L1 (Chitinase-3-like protein 1), is a secreted inflammatory glycoprotein produced by different cell types such as inflammatory cells (neutrophils and macrophages), stem cells, vascular smooth muscle cells, and chondrocytes (6). YKL-40 is also involved in different cellular processes, such as: cancer cell proliferation, survival, and invasiveness, in the inflammatory process around the tumor, angiogenesis, and remodeling of the extracellular matrix (7). High serum or plasma levels of YKL-40 have been detected in patients with various types of cancer. This upregulation has been linked to shorter progression-free and overall survival times in patients with glioma; melanoma; and pancreatic, lung, and breast cancers (8-12).

YKL-40 has been found elevated in serum or plasma in patients with non-cancerous diseases, characterized by inflammation, tissue remodeling, and fibrosis (13). For example in rheumatic diseases (14) and lung diseases such as asthma (15) and chronic obstructive pulmonary disease (COPD) (16). In cardiovascular diseases where YKL-40 has been shown

to be expressed by macrophages in atherosclerotic plaques (17). In patients with diabetes, elevated levels of YKL-40 have been linked to insulin resistance (18).

A previous study revealed that high serum levels of YKL-40 in adult patients with acute myeloblastic leukemia (AML) led to shorter survival times and found that the serum level of this protein was an independent predictor of survival in patients with AML (19). In another study, also carried out in adult patients with leukemia, YKL-40 plasma levels were determined, and it was observed that the YKL-40 levels differ significantly between leukemia patients and healthy individuals (20).

Therefore, YKL-40 protein has received a notable amount of attention as a biomarker of poor prognosis in patients with cancer (7, 13). However, to date, the role of YKL-40 as prognostic biomarker in childhood ALL has not been explored. Therefore, we performed this study to analyze the plasma levels of YKL-40 in Mexican children with ALL and investigate its role as a prognostic factor.

Materials and methods

Study population

We performed a case-control study in a population of children aged 1 to 18 years. The patients were treated at the pediatric oncology clinic of the State Cancer Institute "Dr. Arturo Beltran Ortega" in Acapulco, Guerrero, México, between July 2016 and September 2018. The study involved 45 children who did not receive treatment and who were recently diagnosed with ALL according to the morphological findings of bone marrow aspirate and according to what was reported by Organista, *et al* in 2015 (21).

At the time of admission of each patient, a physical examination was performed to verify the general health signs, genetic and biochemical tests, imaging tests such as ultrasound, nuclear magnetic resonance or computerized axial tomography, which allowed to assess the involvement of other organs Furthermore, each patient included in the study underwent a procalcitonin test, as well as blood and urine cultures to rule out secondary infections. This was considered, to rule out the possible effects of inflammation on YKL-40 levels.

Overall survival was determined as the time between the day of study registration and the day of death (from any cause) or the day of the last contact (21, 22). Survival data were obtained by reviewing the clinical records. Risk classifications in children with ALL were as follows: 1) low risk: children from 1-10 years of age presenting with a leucocyte count of <50,000/mm^3 and 2) high-risk: children <1 and >10 years of age with a leucocyte count >50,000/mm^3.

The patients with ALL were divided into groups; with low and high expression of YKL-40. The median expression of YKL-40 (36.34 ng / ml) was used as the cut-off point to divide the 45 patients with ALL into two groups. Those who expressed YKL-40 at levels below the cut-off value (<36.34 ng / ml) were assigned to the downregulation group (n = 20), and those with expression above the cut-off value (> 36.34 ng / ml) were assigned to the upregulation group (n = 25).

Patients were treated with VDCPM (vincristine, daunorubicin, cyclophosphamide, prednisone, and intrathecal methotrexate) or VDLPM (vincristine, daunorubicin, asparaginase, prednisone, and intrathecal methotrexate) regimens and according to protocol reported by Organista *et al* (21). Following the mentioned treatment schemes and a follow up of approximately 36 months, 64.5% of children with ALL survived and 29.5% died.

The control group was comprised of 45 hematological healthy children aged 1 to 18 years, who visited the Ministry of health, "Dr. Ramón Carreto Leyva", in Chilpancingo, Guerrero, México, for other reasons such as vaccination, weight and height monitoring, and medical consultation. The blood counts in these subjects were measured to confirm normal leukocyte count (4.5–11.0×10^3 leukocytes/mm^3).

Peripheral blood samples from both study groups were used. The parents or guardians of all the subjects signed informed consent forms; the original study was also approved by the ethics committee of the State Cancer Institute of the State of Guerrero. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration.

The blood samples used in both study groups were obtained through venous punctures and were collected in tubes with EDTA as the anticoagulant. Plasma was separated from each sample by centrifugation and stored at -70°C until use.

YKL-40 plasma levels by ELISA assays

YKL-40 plasma levels were measured using ELISAs (CHI3L1 ELISA Kit, Human R&D system, Minneapolis Minnesota, USA), according to the manufacturer's instructions. Plasma samples were diluted 1:10 (90 µL of diluent + 10 µL of plasma). The optical density of each well was determined using a microplate reader (MultiSkan Go; ThermoFisher Scientific, Waltham, MA, USA) at a wavelength of 450 nm. The sensitivity of the ELISA technique was 3.55 pg/mL. All samples were measured in duplicate.

Statistical analysis

Statistical analysis was performed using the SPSS version 25.0 and Prism version 5.0 software. The Mann-Whitney U-test was used to identify significant differences between study populations. Kaplan-Meier curves were used to analyze the effect of YKL-40 plasma levels in the survival of patients with ALL, and the differences were compared using log-rank tests. A univariate logistic regression model was also used to identify factors associated with survival in children with ALL, tested first for sex, presence of cytogenetic alterations such as translocations, risk by age and initial leucocyte count, invasion of SNC, and YKL-40 plasma levels. Those associated factors were included in a second multivariate logistic regression model. A p-value <0.05 was considered statistically significant.

Results

General characteristics of the study population

Overall, 45 plasma samples from children with ALL as well as 45 control samples from healthy children were collected. The characteristics of the children with ALL and the controls are summarized in Table I.

Table I. General characteristics and clinical data of children with and without acute lymphoblastic leukemia (ALL)

Variable	Children with ALL	Children without ALL

	(n=45)	(n=45)
Age (years; average ± SD)	8.7 ± 4.9	9.5 ± 4.0
Leucocyte count/mm^3	24,400 (11,950–63,925)*	6,650 (5,700–8,325)*
Sex	n (%)	n (%)
Male	24 (53.3)	21 (46.7)
Female	21 (46.7)	24 (53.3)
Status of individual		
Alive	29 (64.4)	45 (100)
Deceased	16 (35.6)	-
Leukemia type		
ALL type B	42 (93)	-
ALL type T	3 (7)	-
Presence of translocation	10 (24)	
t(1;19)	2 (4)	-
t(9;22)	5 (11)	-
TAL1	1 (3)	-
t(10;11)	1 (3)	-
t(12;21)	1 (3)	
Absence of translocation	35 (76)	
Risk according to age		
Low risk (1–10 years)	24 (53.3)	-
High risk (<1, >10 years)	21 (46.7)	-
Risk according to leucocyte count at diagnosis		
Low (<50,000 mm^3)	30 (66.7)	-
High (≥50,000 mm^3)	15 (33.3)	-
Invasion of CNS	14 (31.1)	
Without invasion of CNS	31 (68.9)	

*median (percentile 25percentile 75). CNS: Central Nervous System

YKL-40 plasma levels in children with ALL

The median YKL-40 plasma levels in children with ALL was 59.7 ng/mL, which was significantly higher than that in children without ALL (22.5 ng/mL; $p<0.0001$) (Figure 1A).

Clinical characteristics of children with ALL and identification of prognostic factors

The pathological parameters associated with survival in children with ALL in this study are as follows: age, sex, initial leukocyte count, cytogenetic abnormalities such as translocations and invasion of leukemic cells to the central nervous system (CNS).

Univariate and multivariate logistic regression analyses were performed to determine which of the parameters, such as age, sex, presence of translocations, initial leucocyte count, and invasion of CNS in addition to YKL-40 plasma levels, were associated with the survival of children with ALL (Table II). Univariate and multivariate regression analyses revealed that sex and the presence of translocations in this study were not associated with survival in children with ALL. Age, initial leukocyte count, invasion of CNS, and YKL-40 plasma levels were found to be associated with survival in children with ALL.

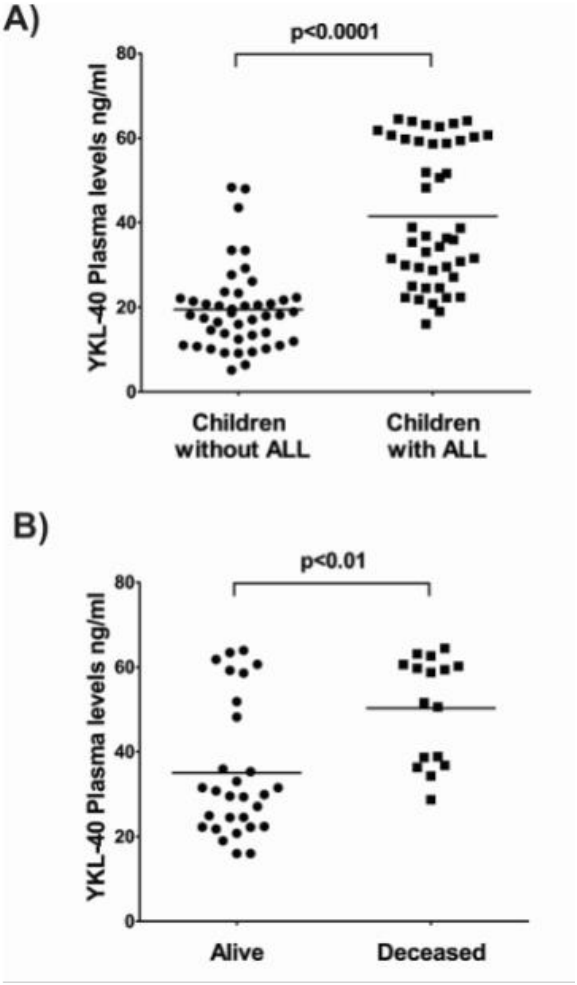

Figure 1. YKL-40 plasma levels in the study population

Figure 1A. YKL-40 plasma levels in children with ALL and children without ALL. Absolute value plot showing YKL-40 plasma levels (ng/mL) in children without ALL (n=45) and children with ALL (n=45). The horizontal solid lines represent the median values. YKL-40 plasma levels in children with ALL is significantly higher (median 59.7 ng/mL) compared to children without ALL (median 22.5 ng/mL) p<0.0001 Mann Whitney U-test. Figure 1B YKL-40 plasma levels in alive and deceased children with ALL. Absolute value plot showing plasma YKL-40 values (ng/mL) in children with ALL who were still alive (n=29, 64.4%) and those who were deceased (n=16, 35.6%). The horizontal solid lines represent the median valuesYKL-40 plasma levels in deceased children with ALL (median 55.1 ng/ml) is

significantly higher compared to living children with ALL (median 31.53 ng/ml) $p<0.01$ Mann-Whitney U-test

High-risk children with ALL (as defined above) were 8.53 times more likely to die than their low-risk counterparts (95% confidence interval [CI] 1.2–58.2 and p=0.03). Children with ALL who had invasion of CNS had a 6.45-fold higher risk of death than did those without invasion of CNS (95% CI 1.01-41.2 and p=0.04). Children with ALL who had high levels of YKL-40 (≥36.34 ng/mL) had a 6.06 -fold higher risk of death than did those with low levels (<36.34 ng/mL) (95% CI 1.1–31.6 and p=0.03,). Low-risk children with ALL who have high plasma levels of YKL-40 (>36.34 ng/ml) could probably have an infection and may also have inflammation patterns characteristic of the disease, leading to increased plasma levels of YKL-40 (Table II).

Table II. Multivariate analysis of prognostic factors in children with acute lymphoblastic leukemia

	Alive n (%) 29 (64.4)	Deceased n (%) 16 (35.6)	Univariate analysis			Multivariate analysis		
			OR	95% CI	P-value*	OR	95% CI	P-value**
Sex								
Male	15 (33.3)	9 (20.0)	1.09	0.59–2.02	0.76			
Female	14 (31.1)	7 (15.6)						
Translocation								
Absence	24 (53.3)	11 (24.5)	2.18	0.52–9.12	0.28			
Present	5 (11.1)	5 (11.1)						
Risk by age and initial leukocyte count								
Low risk[a]	16 (35.5)	3 (6.6)	5.33	1.24–22.8	0.02	8.53	1.2–58.2	0.03
High risk[b]	13 (28.9)	13 (29)						
Invasion of CNS								
Invasion	6 (13.3)	8 (17.8)	3.83	1.01-14.4	0.04	6.45	1.01-41.2	0.04
Without invasion	23 (51.1)	8 (17.8)						
YKL-40 Plasma levels								
Low	19 (42.2)	3 (6.6)	8.2	1.89–35.82	0.005	6.06	1.1–31.6	0.03
High	10 (26.7)	13 (24.5)						

OR=odd ratio, CI: confidence interval. CNS: central nervous system P-value* reference categories were female sex, no presence of translocation, low risk according to age and initial leukocyte count, without invasion of CNS and low YKL-40 plasma levels (<36.34 ng/mL). P-value** were obtained via multivariate logistic regression analysis. P-values <0.05 were considered statistically significant. [a]Low risk include patients with low risk characteristics such as age 1-10 years old and <50,000 leukocyte count. [b] High risk includes patients with high risk characteristics such as age <1 and >10 years old and ≥50,000 leukocyte count.

Association of YKL-40 plasma levels with survival in children with ALL

YKL-40 plasma levels in children with ALL who were alive with the levels in those who died during the study, and found that the median YKL-40 plasma levels in children who had died (55.1 ng/mL) was significantly higher than that in children who were alive (31.53 ng/mL) ($p<0.05$). YKL-40 is a protein that increases when there is inflammation, and it is considered an independent marker. Patients who were alive with high levels of YKL-40 probably had a high inflammatory response owing to which the YKL-40 levels were elevated (Figure 1B).

Children with ALL were followed for approximately 36 months; during this period, 16 (35.5%) died as a consequence of the disease (ALL) and 29 (64.5%) remained alive. The follow up was complete for all the patients. Kaplan Meier analysis revealed a positive association between overall survival and plasma levels of YKL-40. As shown in Figure 2A, children with ALL who had high YKL-40 plasma levels (≥36.34 ng/mL) had shorter survival times than those with low YKL-40 plasma levels (<36.34 ng/mL) (log-rank p=0.008). The cutoff value of 36.34 ng/ml is based on the median value obtained from the YKL-40 plasma levels in children with ALL. Kaplan Meier analysis was performed with variables of poor prognosis in ALL, such as age, sex, presence of translocations, initial leukocyte count and invasion of the central nervous system (CNS). Kaplan Meier analysis revealed a positive association between overall survival and age and invasion to CNS. As shown in figure 2C, Kaplan Meier analysis was performed in children with ALL grouped by age; low risk: children from 1-10 years of age, high-risk: children <1 and >10 years of age, observing that children with ALL younger than 1 year and older than 10 years had a shorter survival than those children with ALL from 1-10 years of age, (log-rank p=0.001). In Figure 2D, children with ALL with CNS invasion had a shorter survival than those children with ALL without CNS invasion (log-rank p=0.032). The variables of sex, presence or absence of translocations and initial leukocyte count were not associated with overall survival of children with ALL in this study (Figure 2B, 2E, 2F).

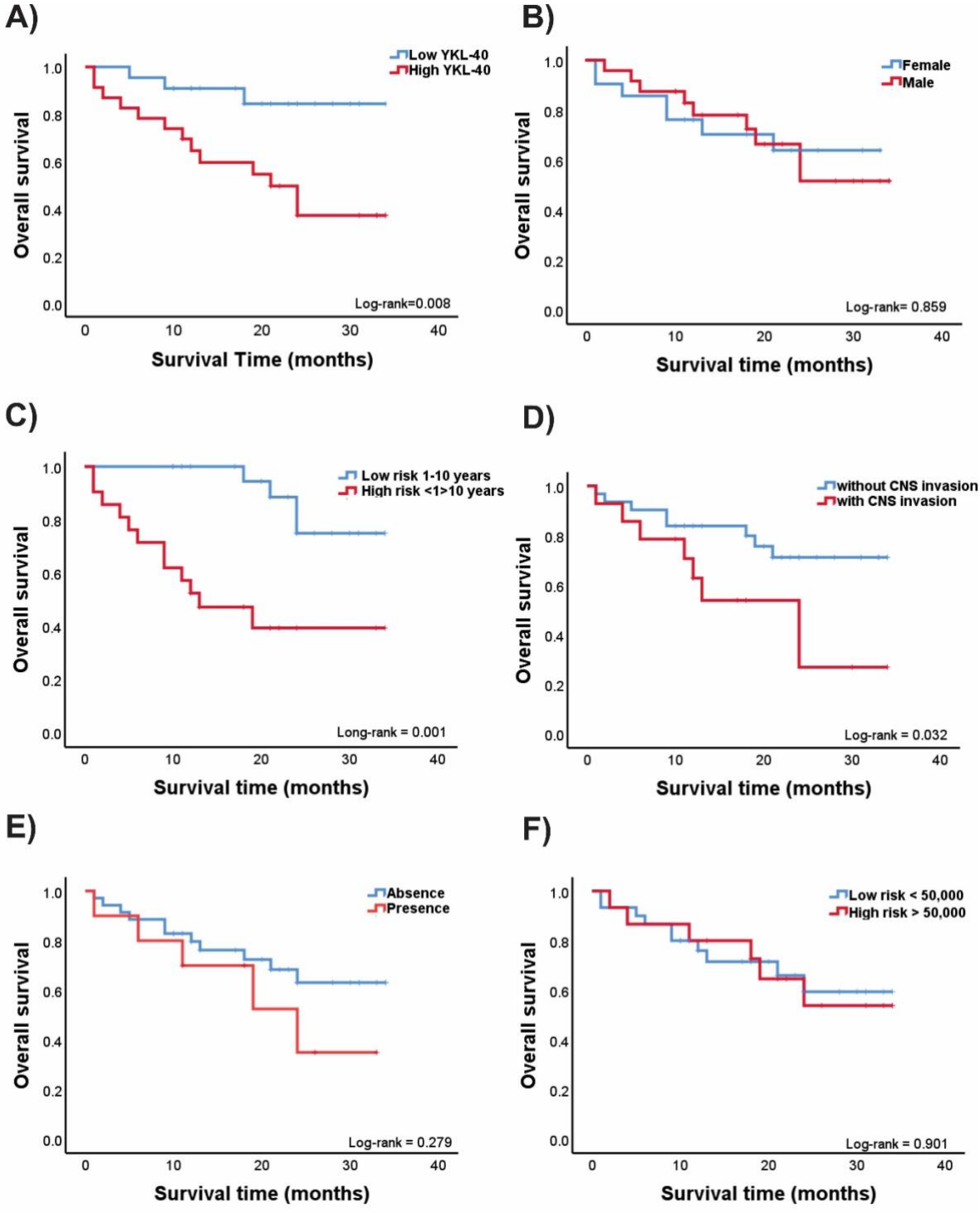

Figure 2. Kaplan Meier curves for overall survival time influence by YKL-40 plasma levels and prognostic factors.

Figure 2A. Kaplan Meier curve for overall survival time influence by YKL-40 plasma levels. Overall survival was significantly shorter for children with high YKL-40 plasma levels (≥36.34 ng/mL) than for those with low YKL-40 plasma levels (<36.34 ng/mL) (p=0.008). Figure 2B. Kaplan Meier curve for overall survival time influence by sex in children with ALL. The variable of sex was not associated with overall survival time in children with ALL (p=0.859). Figure 2C. Kaplan Meier curve for overall survival time influence by age in children with ALL. Overall survival was significantly shorter for children with ALL younger than 1 year old and older than 10 years (high risk group) compared to children from 1-10 years of age (low risk group) (p=0.001). Figure 2D. Kaplan Meier curve for overall survival time influence by Central nervous system (CNS) invasion in children with ALL. Overall survival was significantly shorter for children with CNS invasion compared to children without CNS invasion (p=0.032). Figure 2E. Kaplan Meier curve for overall survival time influence by presence or absence of translocations. The presence of translocations was not associated with overall survival time in children with ALL (p=0.279). Figure 2F. Kaplan Meier curve for overall survival time influence by initial leukocyte count in children with ALL. Initial leukocyte count was not associated with overall survival time in children with ALL (p=0.901).

Discussion

Research has been ongoing to identify new biomarkers that will help determine the prognosis of patients with ALL. Several studies to date have shown that YKL-40 acts as a prognostic biomarker in patients with different types of malignancies, including lung cancer, breast cancer, cholangiosarcoma, and glioma. (23-27). YKL-40 levels were previously evaluated in tissue samples from healthy individuals using immunohistochemistry, where YKL-40 staining intensity was higher in tissues that had high metabolic activity than in those with low activity (28).

In this study, we analyzed the plasma levels of YKL-40 in 45 children with ALL who were between 1 and 18 years of age. The pathological parameters associated with survival in children with ALL are as follows: 1) age: patients younger than one year and older than 10

are considered high risk patients or may have an unfavorable prognosis, 2) sex: female patients have a better prognosis than male patients, primarily due to the appearance of testicular infiltrates that lead to a higher risk of relapse (4,5), 3) initial leukocyte count: an initial leucocyte count of 50,000 leukocytes/mm^3 is the cut off value that determines better or a bad prognosis; patients with a count greater than this cut point are considered high risk (4), 4)cytogenetic abnormalities such as translocations: the presence of t (12; 21) is related to a good prognosis, while t (9; 22) and t (1; 19) are considered to be associated with poor prognosis, since patients who present with these cytogenic abnormalities have a lower rate of resolution of the disease (4, 29) and 5) invasion of leukemic cells to the central nervous system (CNS), which is also considered a poor prognosis factor for the disease (30).

Our statistical analyses suggest that high plasma levels of YKL-40 are associated with shorter survival as well as with poor prognostic factors such as age, leukocyte count at the time of diagnosis, and CNS invasion, and were not associated with other prognosis factors such as presence of translocations and sex. Our statistical analysis also suggests that in addition to the high plasma YKL-40 levels associated with survival, other variables such as age and CNS invasion were associated with survival of children with ALL in this study.

In the present study, we demonstrated that YKL-40 plasma levels was elevated in children with ALL. It is also consistent with other studies that found that high levels of YKL-40 in the serum or plasma are elevated in different types of cancer and solid tumors and were associated with shorter survival durations in patients with cholangiosarcoma, breast, lung, bladder and endometrial cancer (23-27) It has also been found that high levels of YKL-40 were associated with poor prognostic factors; in breast cancer patients with higher tumor-node-metastasis stages was associated with higher tumor-node-metastasis stages (23). High serum levels of YKL-40 were associated with extended (as opposed to limited) disease stage (25) and the International Federation of Gynecology and Obstetrics stage of the tumor in patients with lung cancer and patients with endometrial cancer, respectively (26).

YKL-40 has been shown to increase tumor proliferation, metastatic potential, and angiogenesis (31, 32). At the cellular level, YKL-40 protein expression is high in fetal and embryonic tissue characterized by the morphogenetic changes and marked proliferation and

differentiation (33). No specific cell surface or soluble receptor for this protein has been identified. The growth of fibroblasts derived from osteoarthritic synovial fluid, fetal lungs, and adult skin after stimulation with YKL-40 is similar to that when stimulated by insulin-like growth factor 1 (32). The expression of YKL-40 was found in a variety of tumors and cell lines derived from different types of tumors such as those of bone, brain, breast and lung (34). YKL-40 may play a role in the regulation of the Ras/mitogen activated protein (MAP) kinase pathway, which is one of the best studied signal transduction pathways related to mitogenesis and the conduction of anti-apoptotic and mitogenic signals. YKL-40 has also been linked to MAP kinase and phosphoinositide 3-kinase (PI-3K) signaling cascades in fibroblasts, which leads to the phosphorylation of extracellular signal-regulated kinase-1/2 and Akt-mediated signaling cascades that are associated with mitogenesis and cell survival. Dysregulated activation of these signaling pathways leads to proliferative and antiapoptotic responses that are related to the development of different types of cancer (35). YKL-40 can therefore play a direct or indirect role in the development of leukemia, as it has been reported that activation of the PI-3K/Akt pathway is associated with poor prognosis and drug resistance in pediatric patients with ALL. Inhibition of the PI-3K/Akt pathway leads to a decrease in cell proliferation in chronic lymphoblastic leukemia and AML (36, 37). High expression of YKL-40 in patients with leukemia could promote carcinogenic progression (20). It has been suggested that serum or plasma levels of YKL-40 could reflect some aspects or tumor growth and proliferation and could be used as a biomarker for monitoring of cancer patients during and after treatment (13).

Our results coincide with those published by Hurmale in 2013, where they analyzed plasma levels of YKL-40 in adult patients with leukemia (20), noting that plasma levels of YKL-40 are elevated in patients with leukemia compared to that in healthy individuals. However, in this study the mean YKL-40 plasma levels in patients with leukemia was 168 ng/ml, very different from that obtained in our study, which was 59.7 ng/ml. This may be due to the age of the patients included in our study, (average age 8.7$\pm$ years). YKL-40 levels increase with age and can be related to lifestyle changes (38-40). YKL-40 plasma levels in both sexes are highly correlated with age, which is therefore an important factor to consider when conducting clinical studies on the role of YKL-40 in the prognosis of patients with cancer (41).

Several limitations are important to consider in the present study. This study had a small sample size, which was not enough for validation of the plasma levels of YKL-40 as a prognostic biomarker. It is necessary to increase the number of samples and investigate the specific role of YKL-40 in hematological malignancy. We identified that high plasma levels of YKL-40 are associated with poor prognosis in children with ALL. Therefore, YKL-40 could be a new useful biomarker for actual risk classification in the prognoses of patients with ALL in Mexico and globally.

Conclusions

Our findings provide evidence that high plasma YKL-40 levels, age and CNS invasion are associated with shortened survival in children with ALL. High plasma YKL-40 levels are also correlated with poor prognostic factors such as age, CNS invasion and high initial leukocyte count. This is the first study to clarify the role of YKL-40 plasma levels on the prognosis of children with ALL; however, additional studies with larger cohorts are required to ascertain the role of YKL-40 in this type of cancer. Elevated YKL-40 protein levels could potentially serve as a marker of poor prognosis and shorter survival in children with this hematological malignancy. Nevertheless, our data generate novel hypotheses regarding the YKL-40 levels on survival of ALL patients, which will have to be confirmed in independent studies. In addition, in vitro studies are necessary to determine the role of YKL-40 in acute lymphoblastic leukemia.

Acknowledgments

AARA was the recipient of a PhD scholarship from CONACYT (CVU/Becario: 2366757/211722).This manuscript is part of the doctoral dissertation project of AARA a student of the Programa de Doctorado en Ciencias Biomédicas, Facultad de Ciencias Químico Bilógicas, Universidad Autónoma de Guerrero (UAGro), Chilpancingo, Guerrero, México. The authors thank the State Cancer Institute “Dr. Arturo Beltran Ortega” for the support provided.

Funding

The present study was supported by a grant from CONACYT, México (Fondo Sectorial de Investigación en Salud y Seguridad Social, FSSS01-C-2018-1; grant no. A3-S-47392). This study was also supported by Universidad Autónoma de Guerrero.

Availability of data and materials

Not applicable for that section.

Author Contributions Statement

AARA carry out the experiments, analyzed the data and wrote the paper. YGG, JON, MALV analyzed the data and wrote the paper. MALV, BIA, contributed the reagent and material. EIS, MVSH, MAJL, ABRS provided biological samples and clinical data of patients.

Ethics approval and consent to participate

This study was performed whit the approval of the ethics committee of the State Cancer Institute of the State of Guerrero.

Patient consent for publication

Not applicable for that section.

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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CAPITULO II

Efecto de miR-24 sobre YKL-40 en LLA

Efecto de miR-24 sobre YKL-40 en leucemia linfoblástica aguda

Antecedentes

En México el cáncer es un problema de salud pública al ser la segunda causa de muerte en niños entre 5 y 14 años de edad, con más de 5,000 casos nuevos diagnosticados anualmente, y una prevalencia de 23,000 casos cobrando más de 2,000 vidas anuales. En el año 2014, el cáncer de órganos hematopoyéticos fue el de mayor presencia en menores de 18 años de edad, presentándose el 41% en las mujeres y 59% en los hombres. Las leucemias representan más de la mitad de los casos, siendo la leucemia linfoblástica aguda (LLA) el tipo más común de cáncer (1,2).

Para iniciar un tratamiento quimioterapéutico en un paciente con LLA es importante tomar en cuenta los factores pronósticos conocidos, estos son utilizados en la estratificación inicial de los niños con LLA en grupos de tratamiento (3). Dentro de los factores pronósticos para un paciente con leucemia linfoblástica aguda, se encuentran la edad; los pacientes menores de un año y mayores de 10 años son considerados pacientes de alto riesgo. Otro factor de riesgo es el género; las pacientes de sexo femenino tiene un mejor pronóstico que los pacientes de sexo masculino, esto se debe en parte a la aparición de recaídas testiculares. Conteo de glóbulos blancos; un recuento de 50,000 células/mm^3 es un punto de corte entre un mejor o peor pronóstico. Inmunofenotipo; La determinación del inmunofenotipo de las células leucémicas permite identificar la línea celular afectada, estirpe T o B Los pacientes pediátricos con leucemia aguda de células pre-B o pre-B tempranas responden mejor al tratamiento que aquellos con leucemia de células T y células B maduras y otro factor de riesgo es la presencia de alteraciones citogenéticas como las translocaciones cromosómicas tales como t(12;21), t(9;22), t(4;11), t(1;19) que tienen repercusión en la respuesta al tratamiento (4,5). La búsqueda de nuevos biomarcadores que ayuden al pronóstico de un paciente con LLA debe ser progresiva. Existen estudios que proponen que los microRNAs son una importante herramienta en la clasificación molecular de las leucemias y también se han estudiado como posibles biomarcadores en el pronóstico de la enfermedad (6,7).

Los MicroRNAs son pequeñas regiones de RNA no codificantes de cadena sencilla, de 20 a 22 nucleótidos que regulan la expresión de genes, ya sea por interferir en su traducción o en la estabilidad del RNAm. Los microRNAs comprenden del 1-3% del genoma y regulan la expresión del alrededor del 30% de genes humanos. Los microRNAs provocan el silenciamiento de genes por unirse a secuencias complementarias en la región 3´UTR de RNAs mensajeros blanco (8).

Varios estudios han demostrado que los microRNAs están asociados con múltiples procesos fisiológicos celulares y en procesos biológicos esenciales como el desarrollo, hematopoyesis, envejecimiento y funciones endocrinas (8,9). Funcionan también como reguladores clave en la progresión de un gran número de enfermedades, incluyendo enfermedades del corazón y cáncer. Los microRNAs pueden funcionar como oncomiRs o como supresores de tumor, por tener como blanco a genes supresores de tumor u oncogenes, cuya desregulación lleva al desarrollo de células cancerosas. Estudios realizados han revelado que los microRNAs participan en funciones importantes en la biología del desarrollo del cáncer, incluyendo proliferación celular, apoptosis, angiogénesis, invasión, metástasis y resistencia a fármacos (10-12)

1.1 miR-24

miR-24 es uno de los microRNAs mejor conocido correlacionado con la tumorigénesis, es considerado un onocomiR. El gen que codifica para miR-24 se localiza en el brazo largo del cromosoma 9 región 22.32. miR-24 juega un papel importante en la regulación de la hematopoyesis, apoptosis y proliferación celular (13).

Se ha encontrado sobreexpresión de miR-24 en una gran variedad de canceres, incluyendo cáncer de mama (14), cáncer pancreático (15), cáncer de pulmón de células no pequeñas (16), cáncer gástrico (17) y leucemias (6).

Datos publicados en un metaanálisis en el 2018 sugieren que la desregulación de miR-24 en cáncer, podría predecir una supervivencia global más corta. Con una asociación estadísticamente significativa (P=0.0002) (18).

miR-24 tiene varios blancos que participan en diferentes procesos, algunos blancos de mir24 son; Ciclina A2, CDK4, implicados en el ciclo celular, genes proapoptóticos como FAF-1, caspasa 9 y Apaf-1. miR-24 suprime la expresión de E2F2 y myc, genes implicados en el control del ciclo celular en la diferenciación hematopoyética. miR-24 es sobrerregulado durante la diferenciación terminal de células hematopoyéticas a una gran variedad de linajes, sobre todo en la diferenciación de células T CD8+ (19,20). Estudios realizados en cáncer cervicouterino han propuesto que miR-24 tiene como blanco a YKL-40 (21).

La proteína tipo quitinasa YKL-40 es una proteína filogenéticamente conservada de unión a quitina y heparina, sin actividad quitinasa, es conocida también como glucoproteína 39 del cartílago humano y como proteína 1 tipo quitinasa 3(chitinase-3-like protein 1). El nombre de proteína YKL-40, deriva de su peso molecular que es de 40 KDa y de un código de letras para los aminoácidos presentes en su extremo N-terminal, tirosina (Y), lisina (K) y leucina (L) (22). Contiene una sola cadena polipéptidica de 383 aminoácidos. Es codificada por el gen CHI3L1, localizado en el cromosoma 1 (1q31-q32) (23) y consiste en diez exones de alrededor de 8 Kb. La concentración media en suero de YKL-40 en adultos sanos es de 40 µg/L, no presentando diferencia entre géneros, en personas mayores los niveles de YKL-40 llegan a incrementar (24,25).

Ciertas hormonas y citocinas como IL-6, TNF-α e lL-1β inducen la síntesis de YKL-40 en macrófagos y condrocitos especialmente en condiciones inflamatorias (16). En monocitos humanos revelan que la expresión del RNAm de YKL-40 es fuertemente estimulada por IFN-γ e inhibida por IL-4. Hormonas proinflamatorias como la arginina vasopresina (AVP) y la proteína relacionada con la hormona paratiroidea (PTHrP) afectan la secreción de YKL-40 en cultivo de condrocitos (26).

La proteína YKL-40 es secretada por diferentes tipos celulares, como células inflamatorias (neutrófilos y macrófagos), células madre (células embrionarias y fetales) células musculares vasculares lisas, condrocitos y por células cancerosas de diferentes orígenes (27,28). La función exacta de YKL-40 aún no es del todo clara, sin embargo hay estudios que revelan que juega un papel importante en la proliferación y diferenciación celular (29), angiogénesis (30), inflamación, remodelamiento de la matriz extracelular (31) y apoptosis (32).

Diversos estudios indican que YKL-4 puede jugar diferentes papeles dependiendo del tipo celular y de su microambiente. Se ha sugerido que YKL-40 participa en la proliferación y el desarrollo de células malignas y las protege de sufrir apoptosis, estimula la angiogénesis y tiene un efecto en el remodelamiento extracelular del tejido, regulando la actividad de los fibroblastos e incrementando el grado de fibrosis alrededor de las células cancerosas en tumores sólidos, sin embargo no se ha realizado estudios in vivo que lo demuestren (28).

Niveles elevados de YKL-40 en suero o plasma han sido detectados en pacientes con varios tipos de cáncer como cáncer de pulmón, de mama, de endometrio, colangiosarcoma y se han relacionado con mal pronóstico y corta supervivencia (32-36).

En un estudio llevado a cabo por Sun *et al.,* 2016 en líneas celulares de cáncer cervical, se concluyó que miR-24 tiene como blanco a YKL-40 (21), sin embrago en otros tipos de cáncer como la leucemia linfoblástica aguda no se han llevado a cabo estudios que demuestren que miR-24 tiene efecto sobre YKL-40. Debido a ello en esta investigación se analizó si miR-24 tiene efecto sobre YKL-40 en leucemia linfoblástica aguda.

Material y métodos

Población de estudio y recolección de muestras

Se incluyeron 45 muestras de sangre periférica de niños con diagnóstico confirmado por inmunofenotipo de LLA, que asistieron al servicio de oncopediatría en el Instituto Estatal de Cancerología “Dr. Arturo Beltrán Ortega” (IECAN-ABO) en la ciudad de Acapulco Guerrero, México. Las muestras de sangre periférica fueron tomadas por personal capacitado del laboratorio clínico de dicha institución de Julio de 2016 a Septiembre de 2018.

Con la autorización previa de los padres o tutores mediante la firma de un consentimiento informado, se incluyeron 45 muestras de sangre periférica de niños sin leucemia entre 2 y 17 años de edad, captados en el Centro de salud “Dr. Ramon Carreto Leyva, en la ciudad de Chilpancingo Guerrero, a quienes se les realizó un recuento leucocitario para descartar alteraciones hematológicas. Las muestras biológicas utilizadas en ambos grupos de estudio, fueron muestras de sangre periférica obtenidas a partir de punción venosa, estas se colectaron en un tubo con anticoagulante (EDTA), manteniéndose a 4°C hasta su procesamiento en un lapso máximo de 12 horas. Se separó el plasma de cada una de las muestras y se almaceno a -70°C.

Extracción y purificación de RNA

Los leucocitos totales de las muestras de sangre periférica fueron aislados con buffrer de lisis de eritrocitos (RBL), posteriormente se llevó a cabo la extracción y purificación de RNA total utilizando Trizol® (Invitrogen) de acuerdo con las instrucciones del fabricante. Una vez obtenido el RNA se cuantificó en el equipo NanoDrop 2000c (Thermo Scientific) y posteriormente se llevó a cabo el análisis de la integridad por electroforesis en un gel de agarosa al 1%.

Cuantificación de la expresión de miR-24 y RNAm de YKL-40 por PCR en tiempo real

Para evaluar los niveles de expresión de miR-24, se sintetizó a partir de RNA total, cDNA utilizando el kit de transcripción reversa Taqman MicroRNA (Applied Byosistem Foster City, CA). La mezcla de reacción para la RT contenía 1.5 µL de buffer RT 10X, 0.2 µL dNTPs (100 mM), 0.5 µL de transciptasa reversa MultiScribe (50 U/µL), 0.2 µL de inhibidor de RNAsas (20 U/µL), 3 µL del iniciador RT, 5-10 ng/µL de RNA total en un volumen final de 15 µL. La reacción se incubo en un termociclador (Eppendorf*)* por 30 minutos a 16 °C, 30 minutos a 42 °C, 5 minutos a 85 °C y una etapa final a 4 °C.

La PCR en tiempo real se llevó a cabo utilizando el ensayo Taqman MicroRNA (000402*;* Applied Biosystems*),* 5 µL de TaqMan Universal Master Mix II*,* no UNG (Applied Biosystem), 1 µL de producto de RT, 0.5 µL del ensayo TaqMan MicroRNA*,* en un volumen final de 10 µL. Las condiciones de amplificación utilizadas fueron las siguientes: etapa inicial a 95 °C por 10 min, 40 ciclos a 95 °C por 15 segundos y 60 °C por 1 min*.* Cada muestra se analizó por duplicado y La expresión relativa de los miRNAs sé normalizo con el RNA pequeño nucleolar RNU44 (001094; Applied Biosystems*).*

Para la cuantificación del RNAm de YKL-40, se sintetizó a partir de RNA total, cDNA. La mezcla de reacción para la RT contenía 1 µL de oligo$(dT)_{18}$ (Thermo Scientific), 1 µL de DNTP´s (10 µM), 1 µg de RNA total en un volumen final de 13 µL. La reacción se incubó a 65°C por 5 minutos y 4°C por 1 minuto. Posteriormente se agregó 4 µL de buffer 5x, 1 µL de DTT, 1 µL de RNAsaout (Invitrogen) y 1 µL de enzima SuperScript III (Invitrogen). La reacción se incubó en un termociclador (eppendorf) a 50°C por 60 minutos y a 70°C por 15 minutos.

La PCR en tiempo real se llevó a cabo utilizando el ensayo Taqman Probe spans exons (YKL-40) (Hs01072228_m1 Thermofisher Scientific), 5 µL de TaqMan Universal Master Mix II*,* no UNG (Applied Biosystem), 2 µL de producto de RT, 1 µL del ensayo TaqMan MicroRNA*,* en un volumen final de 10 µL. Las condiciones de

amplificación utilizadas fueron las siguientes: etapa inicial a 95 °C por 10 min, 40 ciclos a 95 °C por 15 segundos y 60 °C por 1 min. Cada muestra se analizó por duplicado y se utilizó como control endógeno para la normalización de los datos el gen Gliceraldehído-3-Fosfato deshidrogenasa GAPDH (Hs03929097_g1 Applied Biosystem).

Para llevar a cabo los ensayos de PCR en tiempo real se utilizó el equipo 7500 Real Time PCR System de Applied Biosystems y el equipo CFX1096 Real time system de BIO-RAD.

El análisis de la PCR en tiempo real tanto para cuantificar los niveles de miR-24 y RNAm de YKL-40, se llevó a cabo con el método de 2- $\Delta\Delta$Ct normalizado para el valor del ciclo umbral (Ct) del control interno.

Cultivo celular

Cultivo primario de linfocitos

Se realizó el cultivo primario de linfocitos, utilizando la técnica de Ficoll (separación celular por gradientes de densidad) que fueron utilizados como control de referencia de la expresión de miR-24 y RNAm de YKL-40. Se procedió a tomar una muestra de sangre periférica en un tubo con anticoagulante (EDTA). Se centrifugó a 2000 rpm por 10 minutos a temperatura ambiente. La fase blanca que se separó, se colocó en un tubo falcon con 3 ml de PBS, se centrifugó 10 minutos a 1600 rpm a temperatura ambiente. Se tomó la capa blanca y nuevamente se colocó en 3ml de PBS + 3 ml de Ficoll, se centrifugó 15 minutos a 1760 rpm con 1 grado de aceleración y sin freno, se separaron tres fases, se tomó la fase de en medio (fase color gris) y se colocó en un tubo Falcon con 3 ml de PBS, se centrifugó nuevamente a 1760 rpm por 15 min con 2 aceleraciones y un freno, se decantó el sobrenadante y se agregaron 2 ml de medio RPMI 1640 sin suero. Se homogenizó suavemente para llevar a cabo el conteo celular, para agregar alrededor de 3, 000,000 de células al frasco de cultivo, posteriormente se agregaron proporcionalmente suero fetal

bovino, fitohemaglutinina y medio RPMI 1640. Los frascos de cultivo se incubaron con 95% de humedad y 5% de CO_2. Al alcanzar un 80% de confluencia celular se procedió a realizar la extracción de RNA y proteínas.

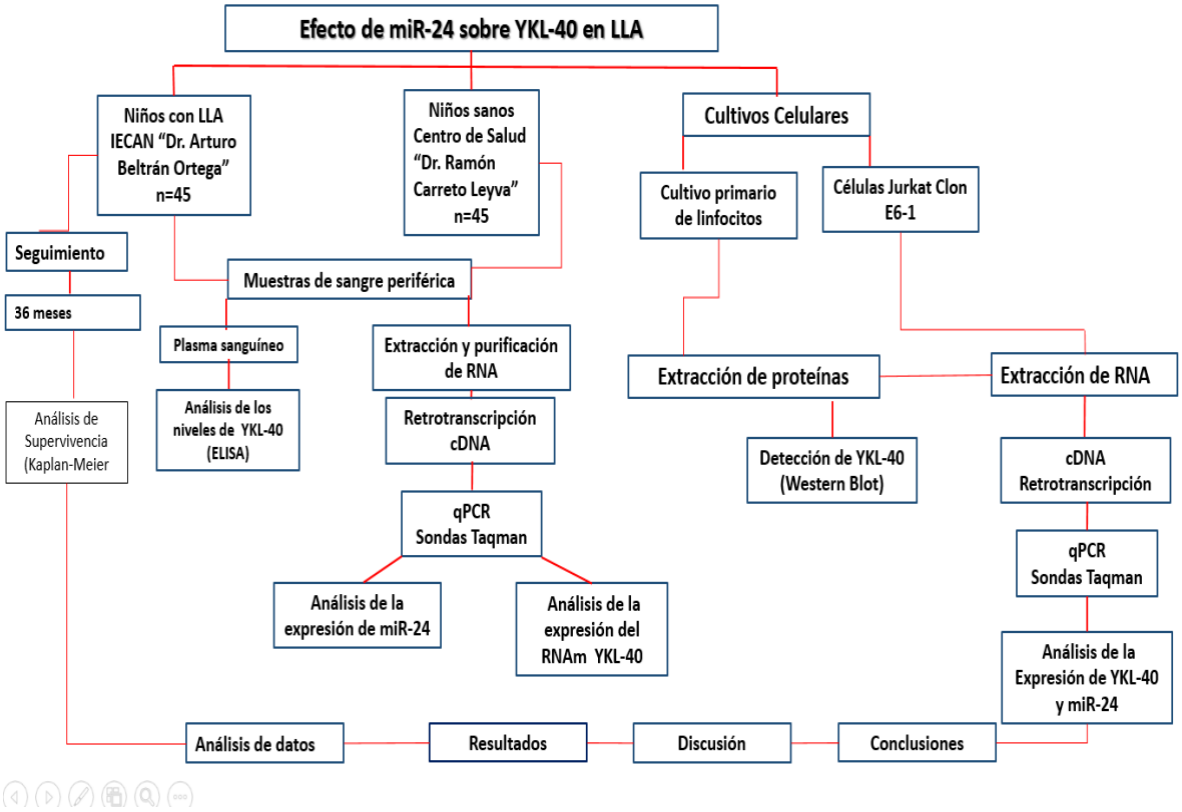
Línea celular Jurkat

La línea celular derivada de leucemia linfoide Jurkat clon E6-1 (ATCC® TIB-152™) se cultivó en medio RPMI 1640, suplementado con suero fetal bovino al 10%, con 95% de humedad y 5% de CO2 y al alcanzar una confluencia celular de 80%, se procedió a hacer la extracción de RNA y proteínas.

Western Blot para medir los niveles proteicos de YKL-40.

Las proteínas (30 µg) se desnaturalizaron utilizando volúmenes iguales de buffer de muestra. Las proteínas se separaron mediante SDS-PAGE al 12.5%, el gel se transfirió a una membrana de nitrocelulosa, la cual posteriormente fue bloqueada por una hora con leche al 5% en PBS 1x. Posteriormente la membrana se incubó con el anticuerpo primario anti-YKL-40/CHI3L1 (ab180569 Abcam, USA) a una dilución 1/500 y α-actina (sc-32251, Santa Cruz, Biotecnhnology, USA) a una dilución 1/3000 a 4°C toda la noche. Las membranas se lavaron 3 veces por 10 minutos con TBST-1x (Tris-HCl 20 mmol/L pH 7.5, NaCl 150 mmol/L y tween 20 0.1%) y posteriormente la membrana se incubó por una hora con anticuerpo secundario anti-rabbit y anti-mousse acoplados a peroxidasa (ThermoFisher Scientific). La membrana se reveló con immobilon western chemilum HRP substrate (WBKLS0100, Merck, USA) de acuerdo a las indicaciones del fabricante. La quimioluminiscencia se detectó usando el Chemidoc XRS+ (BioRad, USA). Las imágenes de WB serán cuantificadas utilizando el software imagen J (versión 1.44 National Institute of Health, Bethesda, MD, USA).

DIAGRAMA DE TRABAJO



RESULTADOS

Expresión de miR-24 en niños con LLA y niños sin LLA

Se evaluó la expresión de miR-24 por RTqPCR en 45 muestras de niños con LLA y en 45 muestras de niños sin LLA, donde se observa un aumento estadísticamente significativo (<0.001) de la expresión de miR-24 en niños con LLA en comparación con niños sin LLA (Figura 1).

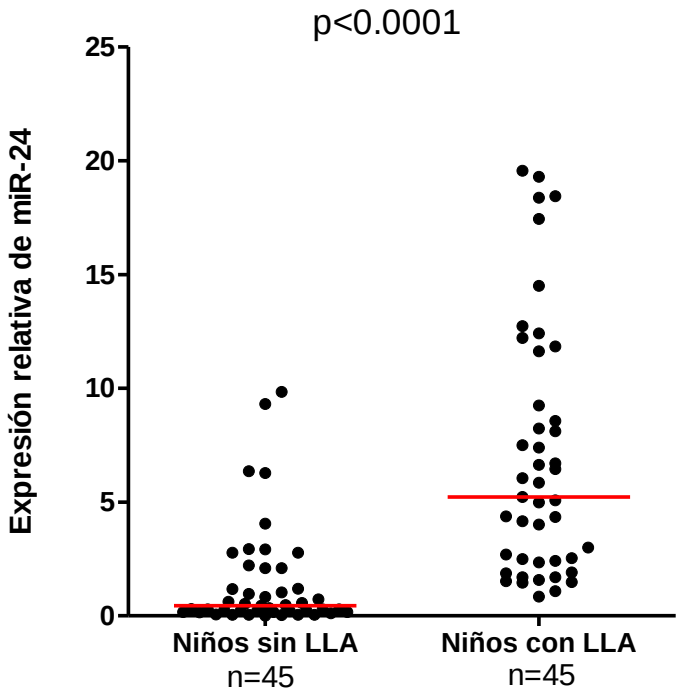


Fig.1 Expresión relativa de miR-24 en niños con LLA y sin LLA. La expresión de miR-24 fue evaluada por RT-qPCR. Los niveles de expresión de miR-24 son significativamente más altos en niños con LLA comparado con niños sin leucemia [niños sin LLA 0.44 (0.16-2.09), niños con LLA; 5.23 (2.38-10.4)]. Los valores están reportados en medianas (línea roja) y (p25 y p75), U Mann Whitney p<0.001.

Expresión del RNAm de YKL40 en niños con LLA y niños sin LLA

Se realizó el análisis de la expresión del RNAm de YKL-40 en 45 muestras de niños con LLA y en 45 muestras de niños sin LLA, observándose alta expresión del RNAm de YKL-40 en niños con LLA comparada con niños sin LLA (p<0.0001) (Figura 2).

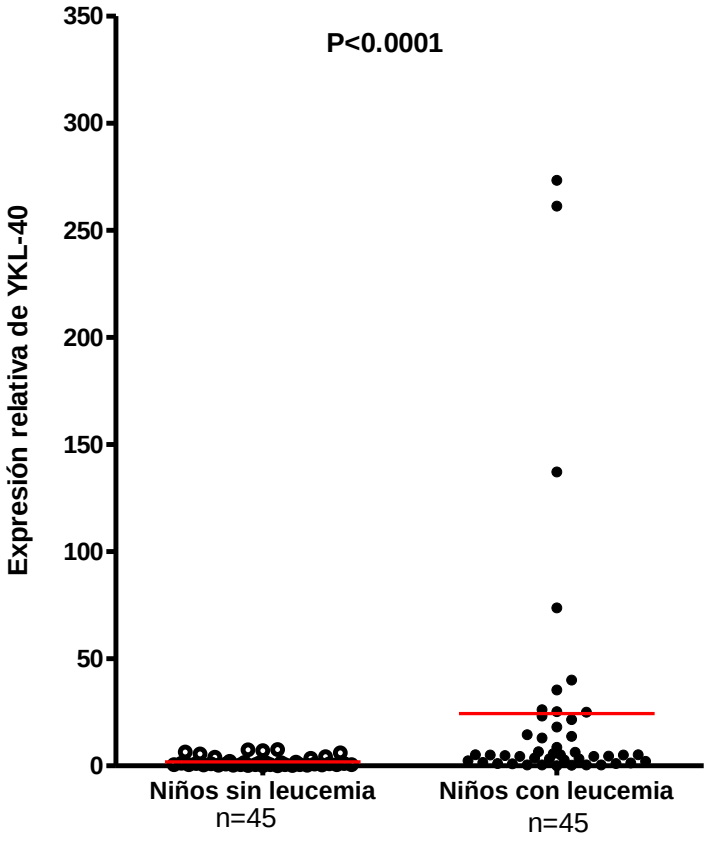


Fig. 2 Expresión relativa de YKL40 en niños sin LLA y en niños con LLA. Cada muestra es representada por un punto. Niños sin LLA 0.85 (0.53-2.04), niños con LLA 5.08 (1.86-19.9). Los valores están reportados en medianas (línea roja) y (p25 y p75), U Mann Whitney p<0.0001.

Supervivencia de niños con LLA y niveles de expresión de miR-24

Se llevó a cabo el análisis de Kaplan Meier para determinar la asociación de los niveles de expresión de miR-24 y la supervivencia de niños con LLA, observando que los niveles de expresión de miR-24 no se asocian con la supervivencia de los niños con LLA p>0.05 (Figura 3).

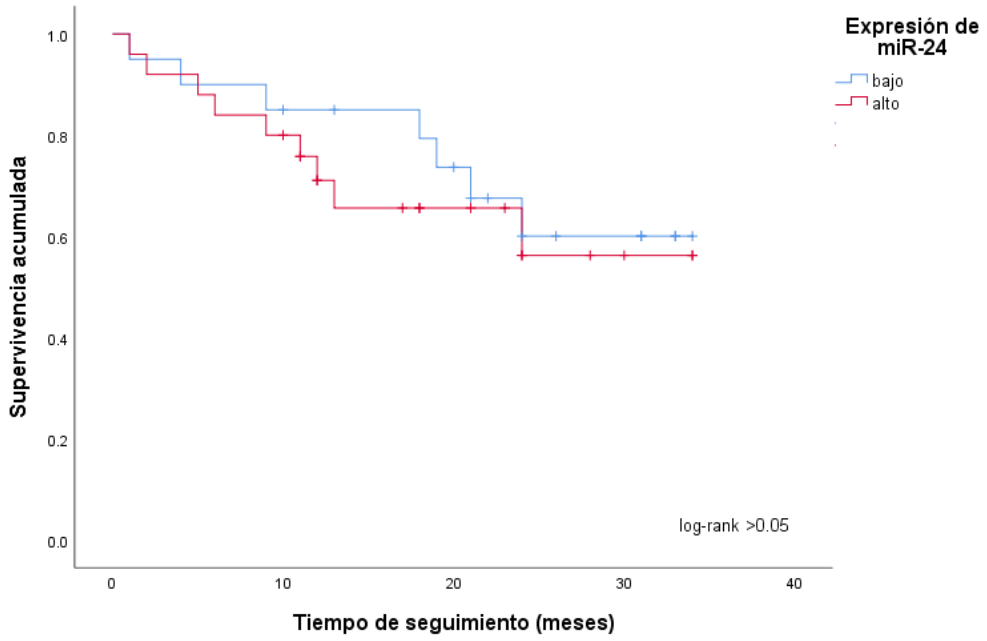


Fig. 3 Análisis de Kaplan Meier de los niveles de expresión de miR-24 y la supervivencia en niños con LLA. La expresión de miR-24 no se asoció con la sobrevida de niños con LLA. (p>0.05).

Supervivencia de niños con LLA y expresión del RNAm-YKL-40

Se llevó a cabo el análisis de Kaplan Meier para determinar la relación entre la expresión del RNAm de YKL-40 y la supervivencia de niños con LLA. No se observaron diferencias en relación a la expresión del RNAm de YKL-40 y la supervivencia de niños con LLA (p>0.005) (Figura 4).

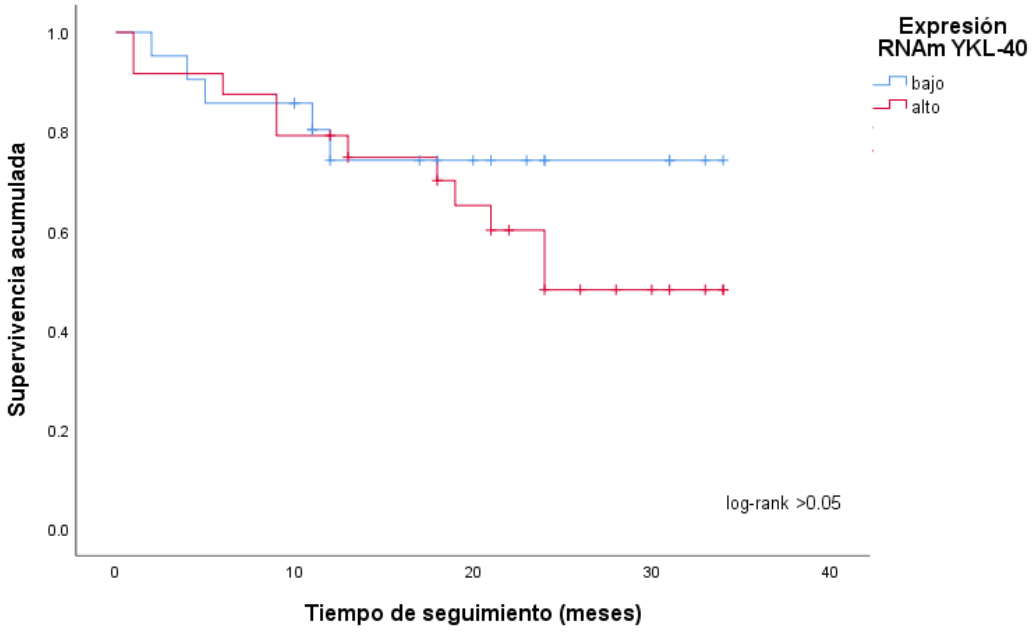


Fig. 4 Análisis de Kaplan Meier de los niveles de expresión de YKL-40 y la supervivencia en niños con LLA. La expresión del RNAm de YKL-40 no se asoció con la supervivencia de niños con LLA. (p>0.05).

Efecto de miR-24 sobre YKL-40 en la línea celular Jurkat clon E6

Se analizó la expresión de miR-24 en cultivo primario de linfocitos y en la línea celular Jurkat clon E6-1, observando que la expresión de miR-24 es mayor en linfocitos comparada con células Jurkat.

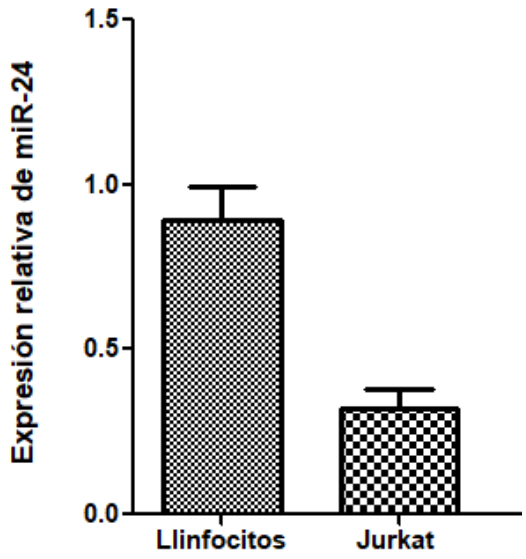


Figura 5. Expresión relativa de miR-24 en cultivo celular. Se analizó la expresión de miR-24 en cultivo primario de linfocitos, en la línea celular Jurkat clon E6-1. La expresión de miR-24 es mayor en el cultivo primario de linfocitos

Se analizaron los niveles proteicos de YKL-40 en cultivo primario de linfocitos, en la línea celular Jurkat clon E6-1 y en la linea celular Nalm6, utilizando como control positivo muestras de CaCu. No se detectó a YKL-40 en cultivo primario de linfocitos células Jurkat y en células Nalm6 (Figura 6).

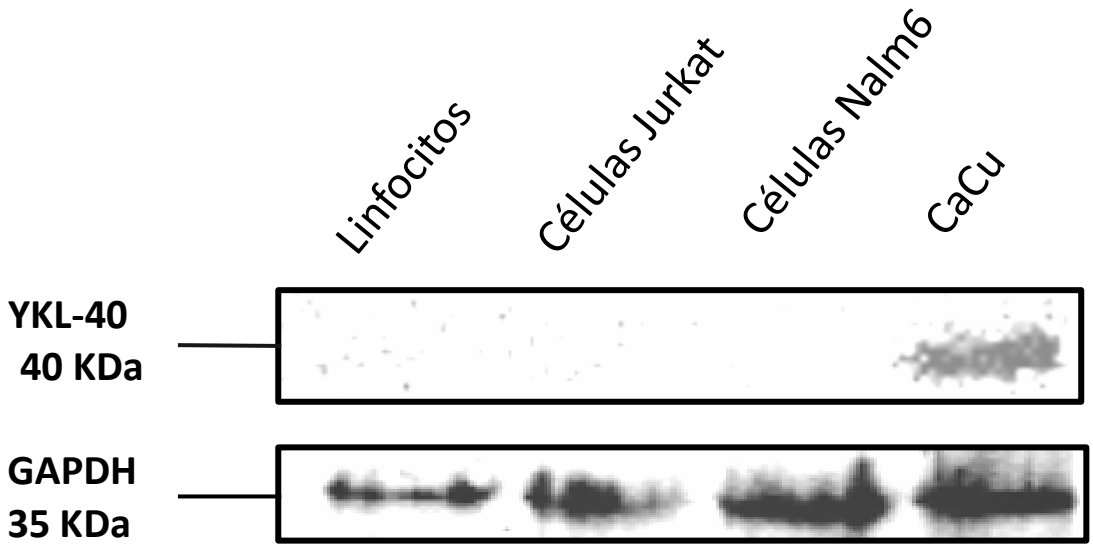


Figura 6. Detección de YKL-40 en linfocitos, células Jurkat clon E6-1 y células Nalm6. No se detectó a YKL-40 en linfocitos, células Jurkat y en células Nalm6

ii. DISCUSION

miR-24 se ha relacionado con la tumorigénesis y ha sido considerado un oncomiR asociado con el desarrollo de cáncer. Se ha encontrado sobreexpresión de este microRNA en diferentes tipos de cáncer como en cáncer gástrico, de mama, de páncreas y en cáncer de pulmón (14-17) estas afirmaciones coinciden con nuestros resultados obtenidos, en donde miR-24 se encuentra sobreexpresado en leucemia linfoblástica aguda.

En este estudio analizamos la expresión de miR-24 y YKL-40 en muestras de niños con leucemia linfoblástica aguda. Los resultados obtenidos demuestran que la expresión de miR-24 es significativamente más alta en niños con LLA comparado con niños sin LLA, estos resultados coinciden con los publicados por Organista en el 2015, en donde la expresión de miR-24 es significativamente más alta en pacientes con leucemia linfoblástica aguda y leucemia mieloblástica aguda en comparación con individuos sanos (6). En un estudio realizado en pacientes con cáncer de mama miR-24 se encuentra sobreexpresado en el plasma y en tejido de pacientes con metástasis comparado con pacientes sin metástasis (37). Esta

sobreexpresión está asociada con etapas avanzadas del cáncer de mama y en pacientes con tumor triple negativo.

Se analizó también la expresión del RNAm de YKL-40 en niños con LLA y en niños sin LLA, la expresión del RNAm fue significativamente más alta en niños con LLA comparada con niños sanos, estos resultados se relacionan con otro estudio realizado en cáncer de vejiga en donde la expresión del RNAm de YKL-40 es significativamente más alta en pacientes con cáncer de vejiga comparada con individuos sanos (38).

En una investigación realizada en cáncer cervical (21), se determinó que miR-24 regulaba a YKL-40 por tener un sitio de unión en la región promotora 3 ´UTR. Los resultados obtenidos en relación a la expresión de miR-24 y YKL-40 en LLA, podemos suponer que miR-24 no está regulando a YKL-40 en LLA, ya que las dos moléculas se encuentran sobreexpresadas en LLA. Podríamos sugerir que la presencia de ciertas moléculas relacionadas con la inflamación podrían estar interfiriendo en que en LLA miR-24 no este regulando negativamente a YKL-40. Las interleucinas como IL-6, TNF-α e IL-1β inducen la síntesis de YKL-40 en macrófagos especialmente en condiciones inflamatorias (26).

La expresión de miR-24 es mayor en cultivo primario de linfocitos comparada con la linea celular Jurkat clon E6-1, estos resultados son opuestos a los observados en un estudio realizado en cáncer cervical, en donde se midió la expresión de miR-24 en células epiteliales cervicales y en la linea celular Caski, observándose que la expresión de miR-24 es significativamente mayor en las células epiteliales cervicales comparada con células Caski ($p<0.0001$). No se detectó a YKL-40 en cultivo primario de linfocitos, en células Jurkat clon E61 ni en células Nalm6. Nuestros resultados son opuestos a los observados en un estudio realizado en cáncer cervical en donde se midieron los niveles proteicos de YKL-40 en células epiteliales cervicales y en la linea celular Caski, observándose que los niveles proteicos de YKL-40 son significativamente más altos en la linea celular Caski ($p<0.0001$) comparado con células epiteliales cervicales (21).

La identificación de receptores específicos y de factores de transcripción específicos para YKL-40 en células leucémicas podría ser blanco de futuros estudios.

iii. CONCLUSIONES

miR-24 y el RNAm de YKL-40 se encuentran sobreexpresado en niños con LLA.

No se observó regulación negativa de miR-24 sobre los niveles de expresión de YKL-40 en LLA.

miR-24 y el RNAm de YKL-40 no se asocian con la sobrevida de niños con LLA.

No se detectó la expresión de YKL-40 en células derivadas de leucemia linfoblástica aguda Jurkat clon E6 y Nalm6.

La proteína YKL-40 en suero de pacientes con LLA se encontró elevada en comparación con niños sin LLA.

Niveles altos de YKL-40 en plasma se asociaron con corta supervivencia en niños con LLA y con factores de mal pronóstico como la edad, invasión al CNS y conteo inicial alto de leucocitos al diagnóstico.

Se requieren realizar más estudios en cohortes más grandes para elucidar el papel que juega YKL-40 en LLA.

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V. ANEXOS

Avens Publishing Group
J Hum Anat Physiol
August 2019 Volume 4, Issue 1

miR-24 and its Role as an Oncogene in Cancer

Abstract

MicroRNAs (miRNAs) regulate the expression of genes involved in numerous cellular processes, including proliferation, metabolism, differentiation and apoptosis. Changes in the expression levels of miRNAs are associated with the development of various diseases, including cancer. Dysregulation of miRNA expression has been demonstrated to have a role in tumorigenesis and it has been revealed that some miRNAs can act either as oncogenes or tumor suppressors in cancer. miRNA-24 has been identified as an oncogene in various types of cancer. This review discusses the function of miRNA-24 as oncogene in gastric cancer, breast cancer, pancreatic cancer, lung cancer and leukemia.

Keywords: microRNA-24; Oncogene; Cancer

Abbreviations

miRNAs: MicroRNAs; DFS: Disease-Free Survival; RFS: Recurrence-Free Survival; SOCS6: Suppressor of Cytokine Signaling 6; BCL2L11 or Bim: BCL2 like 11; EBC: Early Breast Cancer; ING: Inhibitor of Growth; NAIF1: Nuclear Apoptosis Inducing Factor 1; SOX7: Sex-Determining Region Y-box 7

Background

MicroRNAs (miRNAs) are small non-coding RNAs that negatively regulate gene expression by controlling translation or stability of mRNAs via an RNA interference-like pathway. miRNAs comprise 1-3% of the human genome and regulate the expression of 30% of human genes [1]. miRNA sequences can be located within the introns of protein-coding genes or in units that do not encode proteins, and are expressed coordinately. A few miRNA genes are located in gene exons.

One of the main functions of miRNAs is the silencing of gene expression, which occurs through binding of an miRNA to complementary sequences in the 3' Untranslated Region (3'UTR) of target mRNAs [2,3]. Many studies have demonstrated that miRNAs are associated with various cellular functions and important biological processes, such as development, hematopoiesis, aging and certain endocrine pathways. These small molecules also function as important regulators in the progression of many human diseases, including heart disease and cancer [4,5]. miRNAs can have oncogenic or anti-cancer effects by targeting tumor suppressor genes or oncogenes, respectively, whereby dysregulation and dysfunction of the gene expression can lead to the development and proliferation of cancer cells [6]. Recent research indicates that miRNAs have important roles in various aspects of cancer biology, including cell proliferation, angiogenesis, apoptosis, invasion, metastasis and drug resistance [6]. miRNA (miR)-24 is one of the best known cancer-associated miRNAs and it is considered to be an oncomiR. The overexpression of miR-24 has been reported in various types of human cancer, including breast cancer [6], pancreatic cancer, lung cancer [7], gastric cancer and leukemia [8,9].

Open Access Review Article

Journal of
Human Anatomy & Physiology

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Submission: 01 March, 2019
Accepted: 26 July, 2019
Published: 01 August, 2019

Figure 1: Expression of miR-24 and certain target genes in different types of cancer. Overexpression of miR-24 leads to a decrease in the expression of genes such as BCL2L11, SOCS6, ING5, NAIF1 and SOX7 that participate in important processes, including cell proliferation and apoptosis in different types of cancer. miR-24; microRNA-24; BCL2L11, BCL2 like 11; SOCS6, suppressor of cytokine signaling 6; ING5, inhibitor of growth 5; NAIF1, nuclear apoptosis inducing factor 1; SOX7, sex-determining region Y-box 7.

Data published in a meta-analysis from 2018 suggested that alteration in the expression levels of miR-24 in various types of cancer may predict shorter survival [Hazard ratio, 2.49; 95% confidence interval, 1.84-3.37; P<0.0001]. This association was predominantly observed in cancers of the digestive system, in which high expression of miR-24 indicated poor prognosis. This study also analyzed the association of tumor progression, Disease-Free Survival (DFS) and Recurrence-Free Survival (RFS) with the levels of miR-24 expression. This meta-analysis suggested that high expression of miR-24 may be associated with poor DFS, RFS and tumor progression [10].

The aim of this review is to know the role of miR-24 as an oncogene in different types of cancer. For this purpose, a search was made for articles indexed from 2015 to the date in which information

was provided about miR-24 and its role as an oncogene in the carcinogenic process.

We can observe some target genes of miR-24 in different types of cancer (Figure 1). The overexpression of miR-24 leads to decrease in the expression of genes that involves in important processes in the development of carcinogenesis such as cell proliferation and apoptosis. In the following lines we will talk about some of them.

miR-24 in Gastric Cancer

The miR-23a/27a/24-2 cluster is made up of three miRNAs that are considered to be oncogenes in gastric cancer. It has been proposed that this cluster of miRNAs controls the cell cycle, proliferation, differentiation and cell death [11]. miR-23a and miR-24-2 have been reported to be overexpressed in gastric cancer tissue samples. The degree of alteration among miR-23a, miR-24 and miR-27 is variable; however, the overexpression of these miRNAs is consistent [11].

Transfection of gastric cancer cells with miR-23a, miR-24 and miR-27a inhibitors resulted in decreased cell growth. These observations indicate that miR-23a, miR-24 and miR-27a induce tumor development by promoting cell growth [11]. It has been proposed that miR-23a and miR-24 may regulate the progression of gastric cancer by reducing the expression of the target gene, suppressor of cytokine signaling 6 (SOCS6). A decrease in the expression of SOCS6 increases the activation of signals that promote cell proliferation and inhibit apoptosis, which may be associated with the reduced survival of patients with gastric cancer [11]. miR-23a, miR-24 and miR-27 could be useful targets for the development of alternative gastric cancer therapies [11].

In vivo and *in vitro* assays have demonstrated that miR-24 directly targets BCL2 like 11 (BCL2L11; also known as Bim), which is an important regulator of apoptosis. Zhang et al. analyzed the expression of miR-24 and BCL2L11 in gastric cancer tissue samples [8], noting that miR-24 was overexpressed and expression of BCL2L11 was decreased, resulting in increased cell proliferation and migration, and reduced apoptosis. *In vivo* studies have shown that overexpression of miR-24 accelerates tumor growth, while growth is inhibited by BCL2L11 overexpression. miR-24 and BCL2L11 may be important targets for future clinical research in gastric cancer [8].

The aforementioned studies suggest that miR-24 has targets genes involved in important processes associated with the development of gastric cancer (cell proliferation and apoptosis). Two of the genes that may be involved are SOCS6 and BCL2L11. miR-24 could be considered a therapeutic target in gastric cancer, as suppressing the expression or action of miR-24 would result in increased expression of its targets genes, potentially leading to reduced cell proliferation and enhanced apoptosis. These events could improve the prognosis in patients with gastric cancer.

miR-24 and Breast Cancer

miRNAs have an important role in the progression of breast cancer and could serve as useful cancer biomarkers. NanoString technology was used to analyze the expression of 800 miRNAs in plasma samples from patients with primary and metastatic breast cancer, with 29 miRNAs identified to be differently expressed in patients that developed metastasis compared with non-metastatic patients. Of these miRNAs, 24 were overexpressed and 5 were down regulated. miR-24 was overexpressed in the plasma and breast cancer tissue of patients with metastases compared with non-metastatic patients [12]. This overexpression as associated with advanced stage cancer and the triple negative/basal. Patients that exhibited overexpression of miR-24 in primary tumors had a significantly lower survival rate compared to patients with low expression of miR-24 [12]; thus, miR-24 may be a involved in the development of metastasis in patients with breast cancer. This study also analyzed the gene expression in samples low and high miR-24 expression; in the samples with high expression of miR-24, 2,128 genes were upregulated and 1,190 genes downregulated compared with the low miR-24 samples. The differential analysis of gene expression showed that the main deregulated genes had functions in pathways associated with cancer and metastasis, such as survival, migration and proliferation [12]. miR-24 has been linked to the progression of breast cancer in several studies, and has been shown to regulate genes that control DNA damage repair and cell cycle progression [6,12-14].

One of the most important goals in breast cancer research is to identify patients that present with 'Early Breast Cancer (EBC)' as soon as possible and to determine which patients have a higher risk of relapse [13]. In a study by Basova et al. [13], four oncomiRs (miR-24, miR-19a, miR-155 and miR-181) and one tumor suppressor miR (let-7a) were identified in patients with EBC. The expression of these miRNAs was different between EBC patients with high and low relapse rates. Measuring the expression of miR-24, miR-19a, miR-155 and miR-181, and let-7a in patients with EBC could help to establish whether adjuvant therapy is required [13]. miR-155 and miR-24 were measured in serum from patients with Ki-67-positive tumors; these measurements were able to allows predicting the risk of relapse and could be used to help determine the most appropriate therapy for patients with EBC in the future [13]. Furthermore, combining the measurements of oncomiRs levels with the expression of Ki-67 could determine the risk of relapse in patients with EBC [13].

EBC refers to breast cancer that has not yet spread to axillary lymph nodes. Certain factors, including hormone receptors, and markers such as Her2 and Ki67, can help predict the risk of relapse in patients with EBC; however, these methods are imprecise. The search for new biomarkers has indicated that miRNAs could help determine the risk of relapse with more precision. One of the miRNAs that could be useful is miR-24, as expression is low when a tumor is removed; therefore, its re-expression is associated with relapse in patients with breast cancer. Measuring the levels of miR-24 and other miRNAs, such as miR-155, may be independent risk factors that when used together with standardized risk factors might help identify patients with breast cancer that have a high risk of relapse and metastasis development.

The Inhibitor of Growth (ING) genes are a family of tumor suppressor's genes that are involved in several cellular processes, such as apoptosis, cell cycle regulation and chromatin remodeling [15]. ING5 is a member of the ING family with unknown function and regulation; however, downregulation of ING5 expression has been commonly observed in different cancer types [16]. It has been proposed that the expression of ING5 is decreased in breast cancer tissue samples [6]. Cai et al. determined the expression of ING5 after

overexpressing and inhibiting miR-24 in breast cancer cell lines. Cell proliferation was increased and apoptosis was decreased in MCF-7 cells transfected with miR-24 mimics. The opposite effect occurred in transfected MCF-7 cells with miR-24 inhibitors. These results suggest that miR-24 decreases the expression of ING5, leading to an increase in cell proliferation and decrease in apoptosis in cells with breast cancer cells; thus, miR-24 and ING5 have opposing effects on cell proliferation, invasion and apoptosis in breast cancer [6].

miR-24 and Pancreatic Cancer

Pancreatic cancer has a high incidence worldwide, accounting for ~3% of all cancers in the United States, with a very low survival rate (<5%) [17]. miR-24 is overexpressed in pancreatic cancer. Luciferase assays have shown that Bim (also known as BCL2L11) is direct target of miR-24. BCL2L11 belongs to the BCL2 family of proapoptotic genes, however its biological function is not fully established [18].

An increase in miR-24 and decrease in BCL2L11 expression have been demonstrated in pancreatic cancer cells, resulting in cell proliferation and reduced apoptosis [18]. It has been proposed that cell growth, apoptosis and ring formation of vascular endothelial cells are regulated by miR-24 and BCL2L11. Animal models have been used to demonstrate that miR-24 promotes the growth of pancreatic tumors, and that BCL2L11 inhibits tumor growth. A study infected pancreatic cancer cells with lentivirus particles to overexpress miR-24 or BCL2L11, and the cells were then implanted into immunodeficient mice. Tumor size was increased in mice implanted with cells that had high expression of miR-24, and tumor growth was inhibited by overexpression of BCL2L11. These tumor implantation experiments in mice provide strong evidence that miR-24 and BCL2L11 regulate tumor growth in pancreatic cancer [18].

miR-24 and Lung Cancer

Each year, ~1.61 million new cases of lung cancer are diagnosed, with ~1.38 million deaths. Non-small cell lung cancer is the most common lung cancer subtype, accounting for ~85% of cases [19].

Little is known about the role of miR-24 in non-small cell lung cancer. A study by Le et al. identified that miR-24 expression was higher in the serum of patients with lung cancer patients than in healthy controls [7]. Zhao et al. reported that miR-24 was overexpressed in serum samples and tissue samples from patients with non-small cell lung cancer [20]. Additionally, overexpression of miR-24 in serum was associated with shorter survival in patients with non-small cell lung cancer. This study also analyzed the mechanisms by which miR-24 may participate in the development of non-small cell lung cancer, and it was determined that miR-24 acts as oncomiR by regulating the expression of Nuclear Apoptosis Inducing Factor 1 (NAIF1), which results in increased cell proliferation; thus, miR-24 may be a useful a biomarker for the prognosis of non-small cell lung cancer [20].

Yan et al. analyzed the expression of miR-24 and sex-determining region Y-box 7 (SOX7) in lung cancer cell lines [21]. Cell proliferation was analyzed in A549 cells transfected with miR-24 mimics, which increased in cell proliferation, and the opposite effect was observed in A549 cells transfected with miR-24 inhibitors, suggesting that miR-24 increases the proliferation of lung cancer cells [21]. Luciferase assays confirmed that SOX7 is direct target of miR-24-3p. Additionally, miR-24 was shown to increase xenograft tumor growth in mice by targeting SOX7. These results provide evidence that miR-24 may have an oncomiR function in lung cancer by targeting SOX7 [21].

miR-24 and Leukemia

Acute leukemia is the most common childhood cancer. Previous studies have identified the processes by which miR-24 is involved in hematopoiesis and the differentiation of hematopoietic cell lines; however, few studies have investigated the role of miR-24 in acute leukemia. Organista et al. analyzed the expression of miR-24 in patients with acute leukemia [8], noting that patients with acute leukemia had increased expression of miR-24 compared to healthy subjects. Furthermore, the expression levels of miR-24 were higher in patients with acute myeloid leukemia than in patients with acute lymphoid leukemia.

It has also been reported that there is a significant correlation between miR-24 expression levels and the risk of relapse in patients with acute leukemia. Patients with high expression of miR-24 have a significantly higher risk of relapse compared to patients with low expression of miR-24. Furthermore, acute leukemia patients with high expression of miR-24 tend to have shorter survival compared to patients with low expression of miR-24 [9]. Thus, high expression of miR-24 is associated with poor prognosis and short survival in patients with acute leukemia. miR-24 may be an independent prognostic marker that is able to determine the clinical behavior of patients with acute leukemia [9].

Conclusion

Dysregulation of miRNAs expression has been reported to be associated with carcinogenesis, invasion and metastasis. miRNAs can be detected and measured in serum, plasma and tissue from patients with cancer, and thus be used as biomarkers; however, more research is required so that they can be used clinically. Previous studies have revealed associations between the levels of expression of various miRNAs and the prognosis of cancer patients.

miR-24 is an miRNA that is considered to be an oncomiR as it is overexpressed in different types of cancer, such as gastric cancer, where it decreases the expression of SOCS6 resulting in increased cell proliferation and decreased apoptosis. In breast cancer, the overexpression of miR-24 is associated with the development of metastasis and the risk of relapse. It is also associated with the regulation of genes such as ING5 that control the cell cycle, proliferation and apoptosis in this type of cancer. In pancreatic cancer, the overexpression of miR-24 leads to the decrease in BCL2L11 expression, and in lung cancer, miR-24 decreases NAIF1 and SOX7 expression, which alters cell proliferation and apoptosis. Finally, in leukemia overexpression of miR-24 is associated with poor patient prognosis and reduced survival duration.

All the findings discussed above indicate that miR-24 regulates the expression of genes involved in key processes of carcinogenesis, such as cell proliferation and apoptosis. The overexpression of miR-24 may be a predictor of poor prognosis and, together with the measurement of other established markers for each type of cancer, could be used to help improve the survival and prognosis of patients with cancer.

Perspectives

A clear understanding of the mechanisms by which miR-24 acts as oncogene could allow miR-24 to be used as a prognostic marker; however, further studies are required corroborate this.

More basic and clinical research is required to fully understand the regulation and role of miR-24 in cancer. With a clear understanding of the molecular mechanisms that regulate miR-24, this miRNA could potentially be used as a therapeutic target for different types of cancer, as it has been established that miR-24 is involved in the regulation of genes that mediate key signaling pathways during the development of metastasis. The measurement of miR-24 expression levels could help to identify patients at high risk of relapse and those with poor prognosis.

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