4. HISTOPATOLOGÍA

Como otras neoplasias cutáneas, el SK se desarrolla a través de diferentes estadios evolutivos, observándose una considerable variación en la histopatología dependiendo de la fase del desarrollo en la que se encuentre la enfermedad. Sin embargo, no parece haber diferencias en la apariencia histológica de las lesiones de SK entre los distintos grupos clínicos. En general, el espectro histológico del SK se divide en tres estadios paralelos a las fases clínicas de la enfermedad: el estadio macular, el estadio pápula-placa y el estadio nodular o tumoral.

Existe un solapamiento entre las fases de desarrollo de la enfermedad, ya que puede identificarse simultáneamente en un mismo paciente características histológicas de diferentes estadios del SK en lesiones con distinta localización, o incluso en una misma lesión.

Asimismo, los hallazgos histopatológicos descritos para las lesiones maculares iniciales pueden observarse también en áreas de piel clínicamente sana de pacientes con SK, hecho que apoya el carácter difuso de este proceso desde sus inicios (Weiss 2001).

Aunque algunos autores han descrito diferencias entre el aspecto histológico del SK asociado a SIDA y el clásico, estas se encuentran relacionadas probablemente con los cambios sutiles observados en las lesiones incipientes del SK-SIDA (Niedt 1990).

4.1. ESTADIO MACULAR

El estadio macular del SK se caracteriza por cambios histológicos discretos y poco prominentes, ya que histológicamente las lesiones iniciales del SK son de naturaleza predominantemente inflamatoria y vascular, y suelen confundirse con dermatosis inflamatorias (Ackerman 1999). Por ello, muchas veces la apariencia microscópica inicial puede no ser diagnóstica.

En las máculas iniciales existe generalmente un infiltrado inflamatorio crónico escaso y parcheado, localizado en la dermis superficial, que se distribuye a nivel perivascular y en la periferia de las lesiones vasculares. Este infiltrado está constituido predominantemente por células plasmáticas, linfocitos y macrófagos, algunos de ellos cargados de hemosiderina (hemosiderófagos).

Por otro lado, el estadio inicial de mácula también se reconoce a bajo aumento por un sutil incremento en el número de vasos del plexo vascular superficial **(Imagen 7)**. A mayor aumento, los vasos normales, caracterizados por contornos redondeados, se asocian a una proliferación de pequeños canales vasculares angulados y de morfología irregular. Estos canales vasculares muestran unas paredes finas revestidas por células endoteliales aplanadas y focalmente prominentes.

Dado que el plexo vascular superficial se extiende alrededor de los anejos dérmicos, este proceso proliferativo se localiza también alrededor de los folículos pilosos y de las glándulas sebáceas. Al ramificarse, los canales vasculares separan los haces colágenos

disecando el estroma dérmico. Asimismo, los vasos neoformados muestran una tendencia a rodear a vasos sanguíneos y estructuras anexiales preexistentes, ofreciendo una imagen en la que parece como si estas estructuras fueran en realidad las que improntan a los vasos neoformados. Esta apariencia característica ha sido denominada como el signo del promontorio **(Imagen 8)**, y aunque es común en el SK, no es específico (Gottlieb 1982).

En las lesiones maculares tardías el infiltrado vascular es más extenso, constituyendo en la dermis superficial una red de vasos aserrados y anastomosados entre sí. En este estadio, es frecuente la extravasación de hematíes y la presencia de abundantes hemosiderófagos.

En algunas lesiones maculares se identifican ocasionales células fusiformes proliferantes en el intersticio de la dermis papilar, cerca del plexo vascular superficial, y en los plexos vasculares que rodean a la porción secretora de las glándulas sudoríparas, sin apreciarse en ningún caso conexión aparente con los vasos sanguíneos normales. Con todo, el componente fusocelular en este estadio es casi siempre escaso (Elder 1997; Weedon 1997).

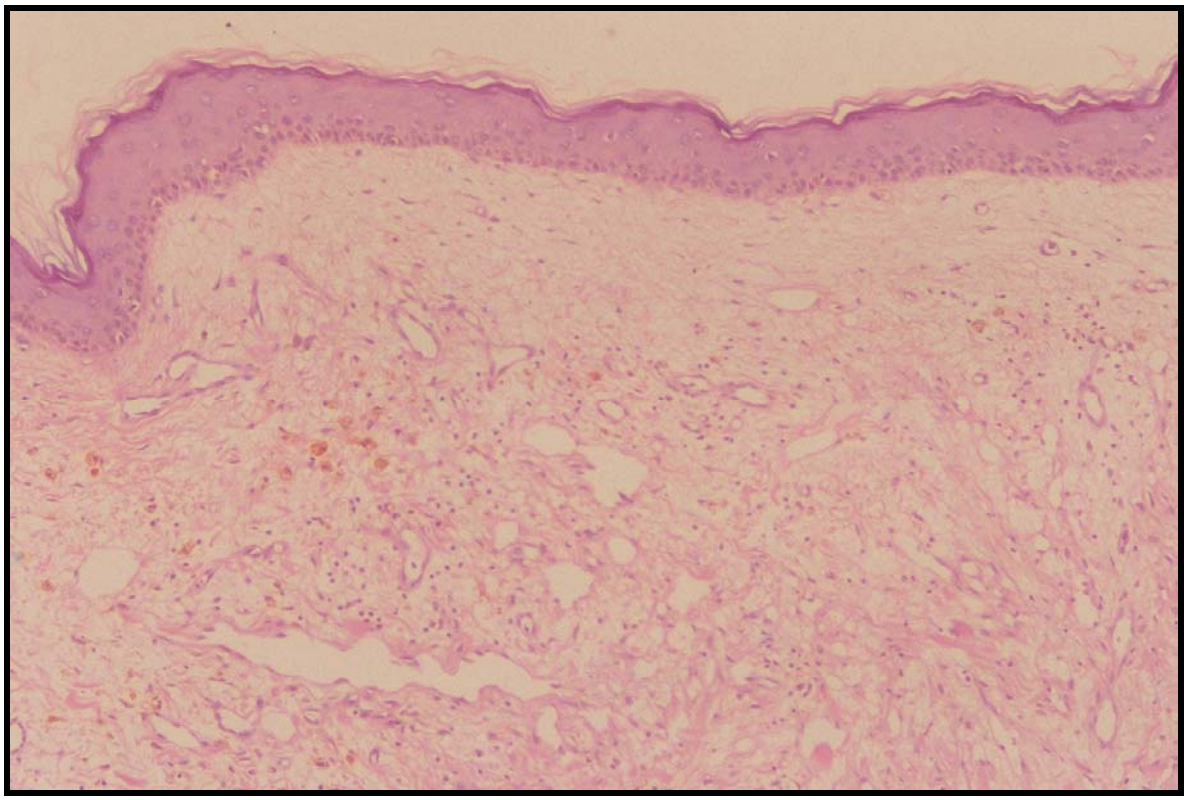

Imagen 7: Estadio de mácula de un varón con SK clásico. Se aprecia a nivel de la dermis superficial un incremento de la densidad vascular, asociado a la presencia de hemosiderófagos y un discreto infiltrado inflamatorio crónico.

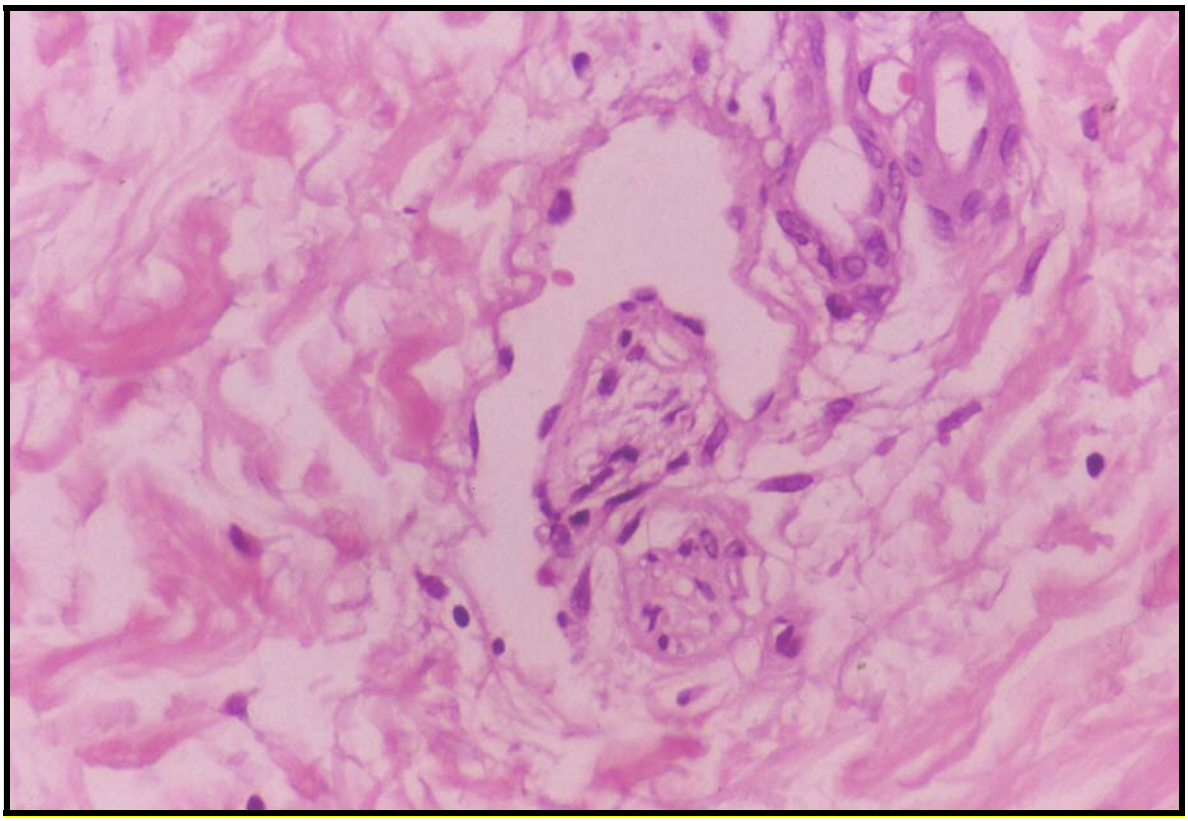

Imagen 8: Estadio de mácula. Un vaso neoformado del SK rodeando a una terminación nerviosa dérmica preexistente (signo del promontorio).

4.2. ESTADIO DE PÁPULA-PLACA

En un estadio más avanzado de la enfermedad, las lesiones del SK suelen afectar a toda la dermis e incluso a la porción superficial de la grasa subcutánea. Este hecho se traduce clínicamente por unas discretas elevaciones de la piel denominadas pápulas. Posteriormente, estas lesiones se extienden progresivamente y coalescen entre sí formando placas sobreelevadas.

A nivel histológico las pápulas y las placas del SK están constituidas por múltiples canales vasculares dilatados y angulados **(Imagen 9)**, con tendencia a anastomosarse entre sí y a disecar los haces colágenos dérmicos. Estos espacios vasculares se encuentran revestidos por un endotelio atenuado y pueden mostrar una morfología variable: algunos forman cordones pobremente canalizados, otros vasos ovoides que contienen hematíes y, por último, algunos adquieren incluso una morfología de aspecto linfático.

Es típico que en este estadio se haga discernible un componente de células fusiformes prominentes y sin atipia, distribuidas laxamente de forma aislada, en pequeños fascículos o en cordones sólidos. Inicialmente se localizan agregándose alrededor de los canales vasculares proliferantes y posteriormente entre los haces colágenos, donde estas células delimitan entre sí unos espacios minúsculos y groseros, a modo de hendiduras vasculares irregulares, que pueden contener eritrocitos. Es por tanto característico del estadio pápula-placa del SK un patrón tumoral bifásico: angiomatoso -canales y hendiduras vasculares- y sólido -proliferación fusocelular- **(Imagen 10)**.

El infiltrado inflamatorio acompañante es más prominente que en la fase de mácula, aunque está constituido básicamente por el mismo tipo de células inflamatorias, es decir, células plasmáticas y linfocitos (Elder 1997; Weedon 1997) **(Imagen 11)**.

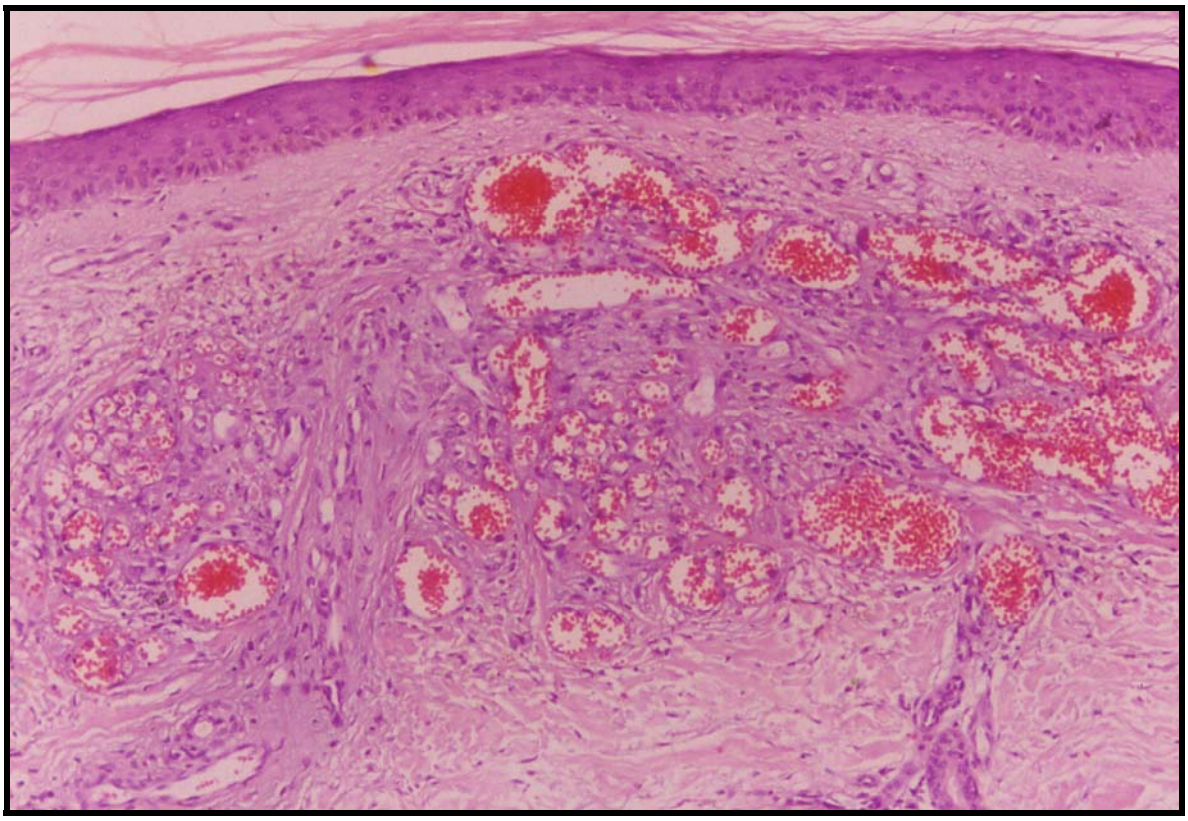

Imagen 9: Estadio de pápula-placa en una mujer con SK clásico. Abundantes canales vasculares dilatados y de morfología irregular.

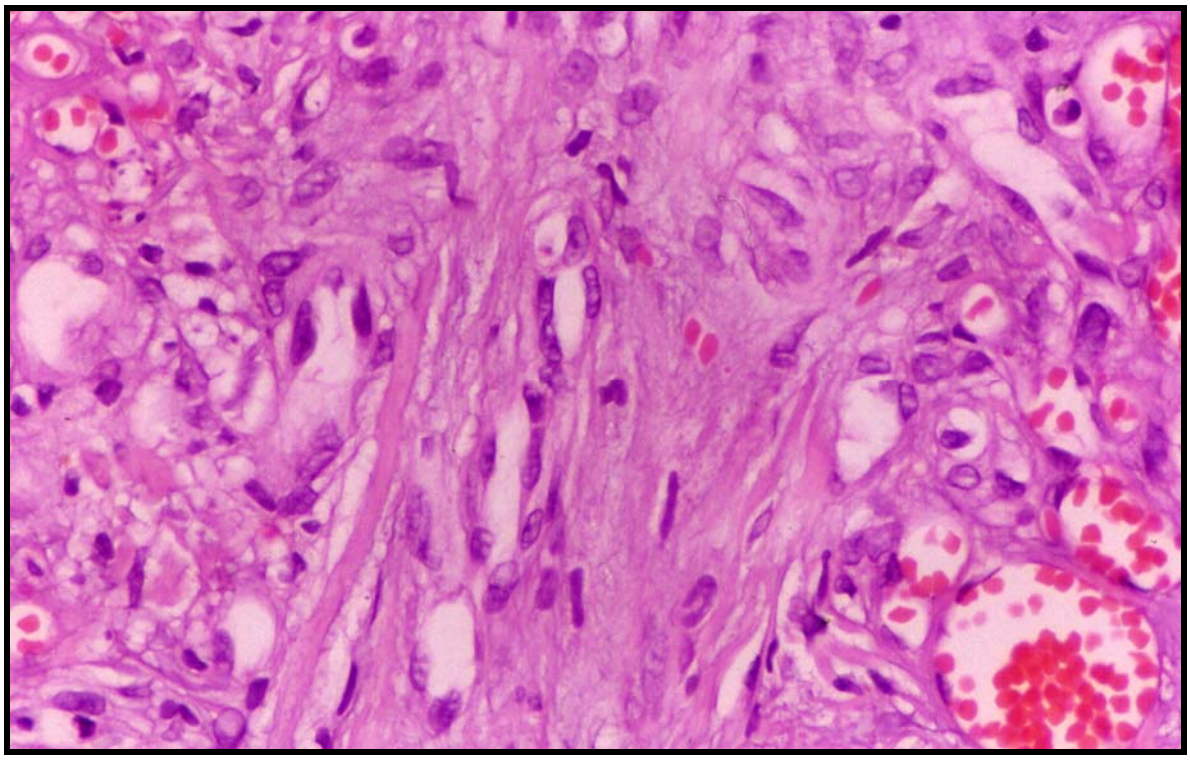

Imagen 10: Estadio de pápula-placa. Patrón bifásico angiomatoso y fusocelular típico.

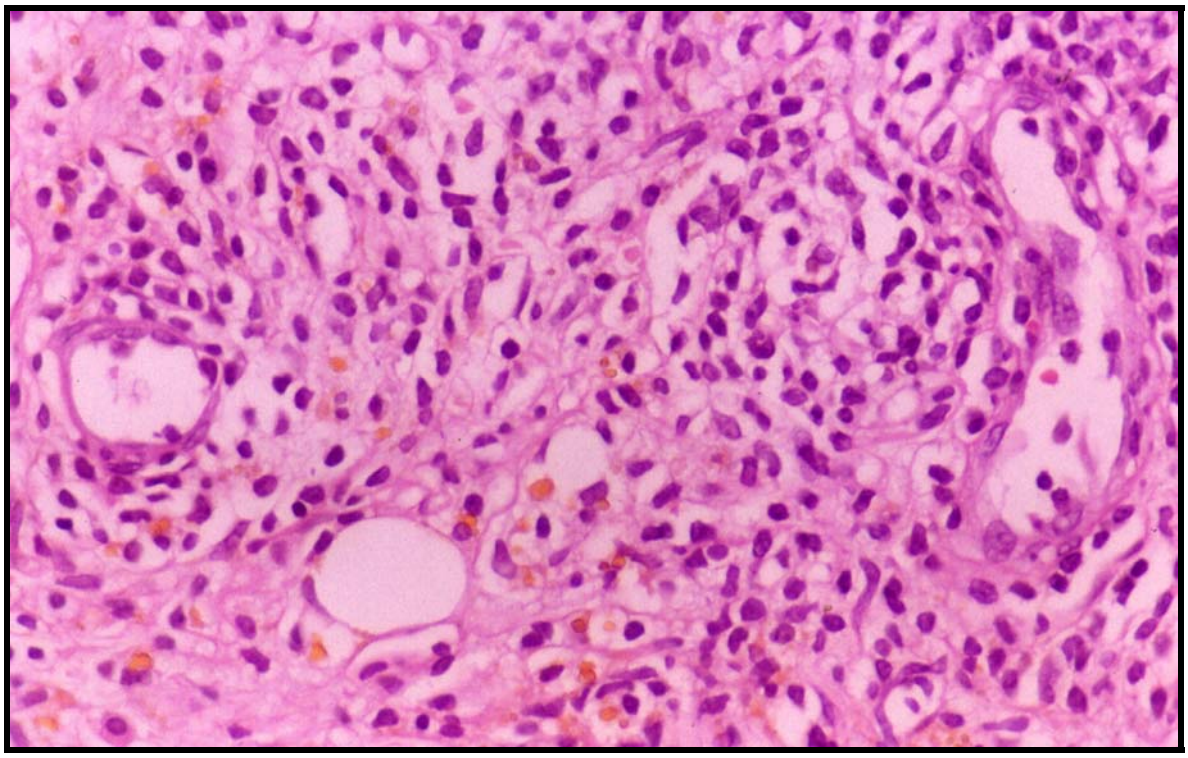

Imagen 11: Estadio de pápula-placa. Moderado infiltrado inflamatorio crónico linfoplasmocitario perivascular e intersticial. Presencia de abundantes hemosiderófagos.

4.3. ESTADIO NODULAR O TUMORAL

Finalmente, el estadio tumoral se caracteriza por la presencia a bajo aumento de nódulos celulares bien definidos, localizados en la dermis **(Imagen 12)** y en el tejido subcutáneo, y separados por bandas de tejido fibrocolágeno. Estos nódulos están constituidos por una proliferación de haces entrelazados de células fusiformes que forman agregados celulares o nidos expansivos, que a su vez delimitan los característicos espacios vasculares en hendidura **(Imagen 13)**. Estos espacios, que frecuentemente muestran eritrocitos en su interior, se han creado a partir de la alineación paralela de las células fusiformes proliferantes. Aunque generalmente ambos elementos están presentes, focalmente cualquiera de ellos puede predominar sobre el otro.

Es decir, progresivamente y a medida que avanza el estadio de la enfermedad, el número de células fusiformes se incrementa, adquiriendo un patrón de proliferación fascicular constituido por haces y fascículos entrelazados entre sí. Estos focos de proliferación fusocelular coalescen produciendo las clásicas lesiones nodulares del SK. Estas células fusiformes son elongadas y con un citoplasma bien definido. El núcleo es ovalado y a veces algo aplanado, con una cromatina finamente granular y un nucléolo poco prominente. Aunque la atipia nuclear es leve o inexistente, las figuras mitóticas son más frecuentes que en las fases previas de la enfermedad.

Es particularmente característico que las hendiduras vasculares anteriormente descritas estén revestidas por células endoteliales delicadas y aplanadas que suelen pasar desapercibidas en las tinciones histológicas de rutina (hematoxilina-eosina). Asimismo, es

también típico que estas hendiduras vasculares se encuentren tan cercanas entre sí que parezcan estar en contacto directo. Este fenómeno, denominado en terminología anglosajona "back-to-back" (espalda con espalda) puede utilizarse como un criterio en el diagnóstico diferencial con otras entidades. Por ejemplo, en los espacios vasculares del granuloma piógeno y de muchos angiomas, los vasos se encuentran más separados por el estroma, estando raramente en contacto, y siendo las células endoteliales de las paredes vasculares más prominentes (ver apartado 5).

En general, la presencia de espacios vasculares y células fusiformes en estrecho contacto entre sí, constituyendo un complejo patrón a modo de panal de abeja es quizás el hallazgo histológico más relevante del estadio nodular del SK (Elder 1997; Weedon 1997; Weiss 2001) **(Imagen 13)**.

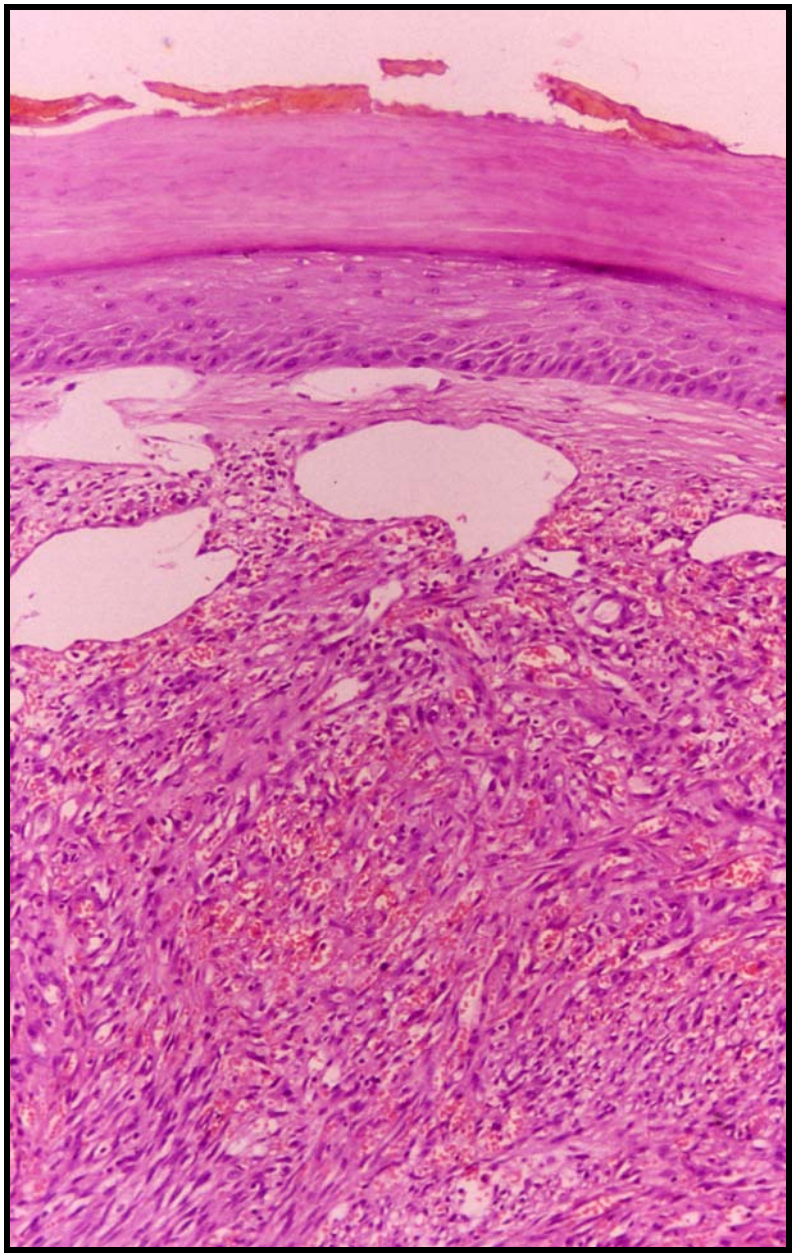

Imagen 12: Estadio tumoral del SK en un varón con SK asociado a SIDA. Proliferación fusocelular nodular densa dérmica asociada a canales ectásicos periféricos.

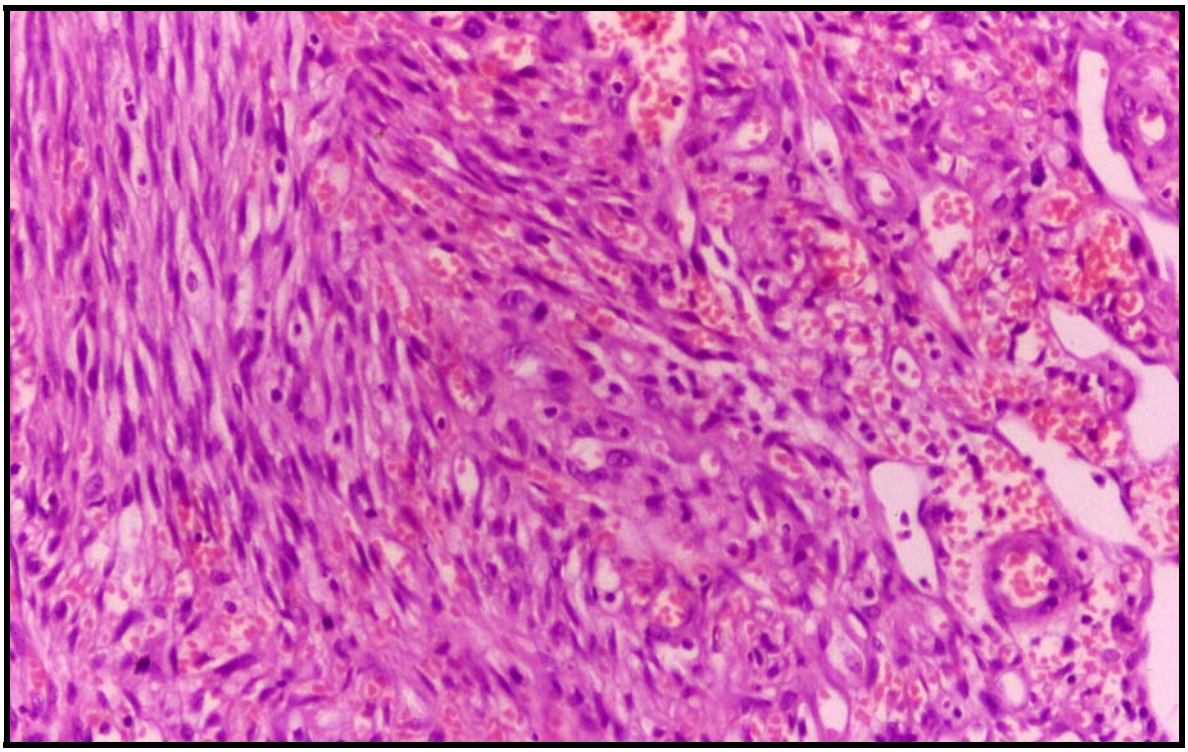

Imagen 13: Estadio tumoral. Las células fusiformes adquieren un patrón de crecimiento fascicular, delimitando espacios vasculares en hendidura. Nótese la estrecha relación entre los espacios vasculares y las células fusiformes.

4.4. CARACTERÍSTICAS CELULARES

En el SK puede observarse atipia celular, pleomorfismo y figuras mitóticas, aunque normalmente no son fenómenos prominentes. De forma general, en los estadios más iniciales del SK las figuras mitóticas son ocasionales y el pleomorfismo celular está prácticamente ausente. Ambos se incrementan progresivamente a medida que se avanza en el estadio de la enfermedad, aunque siempre dentro de un rango leve-moderado. En raros casos, especialmente en la variante africana o en formas agresivas de la enfermedad, pueden identificarse un número significativo de mitosis, así como un marcado pleomorfismo y atipia nuclear.

4.5. OTROS COMPONENTES HISTOPATOLÓGICOS

En mayor o menor medida, en todos los estadios del SK está siempre presente un infiltrado inflamatorio constituido por linfocitos, células plasmáticas, histiocitos, macrófagos, hemosiderófagos y esporádicamente neutrófilos. La presencia de células plasmáticas alrededor de los vasos sanguíneos irregulares neoformados parece ser una característica clave en el diagnóstico histopatológico de las lesiones iniciales del SK, ya que en la mayoría de las dermatosis cutáneas las células plasmáticas raramente se detectan (Gottlieb 1982).

Debe remarcarse que además de los canales vasculares y las hendiduras resultantes de la proliferación de células fusiformes, también se identifica en la periferia de la lesión la formación de numerosos vasos ectásicos que corresponden a capilares sanguíneos regulares (estadios iniciales) o incluso vasos de pared gruesa (estadios avanzados) mezclados entre sí o bien dispuestos alrededor de las lesiones del SK. Probablemente representan vasos reactivos o nutricios, y no un componente básico del tumor (Weiss 2001).

La presencia de eritrocitos extravasados y hemosiderófagos esparcidos dentro y alrededor de la lesión es casi siempre un hallazgo prominente en la cercanía de las lesiones del SK. La eritrofagocitosis -fagocitosis de eritrocitos extravasados por macrófagos tisulares y células tumorales- ha sido descrita en todos los estadios del SK. Otra característica es la presencia, a nivel óptico y ultraestructural, de ocasionales células endoteliales necróticas o apoptóticas (McNutt 1983; Niedt 1990; Chor 1992). Se trata de un hallazgo que se ha

observado frecuentemente en las placas y en los nódulos, y en menor grado en las lesiones maculares.

Los glóbulos hialinos eosinófilos son grupos de esférulas eosinófilas, de morfología parecida a los cuerpos de Russell, que miden entre 1 y 10 μm y que pueden encontrarse en las lesiones del SK, tanto a nivel intracelular (células fusiformes y macrófagos) como extracelular. Son positivos para la tinción de PAS (diastasa resistentes), para la tinción del tricrómico de Mallory -rojo brillante-, y también son autofluorescentes. Su naturaleza es incierta: mediante estudios ultraestructurales y técnicas histoquímicas (positividad para el azul de toluidina y para la peroxidasa endógena) se han hallado membranas degeneradas de hematíes en los fagolisosomas de las células fusiformes y de los macrófagos, por lo que probablemente representan eritrocitos parcialmente digeridos.

Los glóbulos hialinos se describieron por primera vez en casos africanos, aunque también se han identificado en el SK clásico y en la forma epidémica asociada a SIDA, siendo más abundantes en esta última. Pese a que son más comunes en las lesiones en placa y nodulares del SK, pueden estar presentes en cualquier estadio de la enfermedad, observándose ocasionalmente en las lesiones iniciales. Aunque no son enteramente específicos del SK, dado que también han sido descritos en otras entidades donde existe proliferación vascular y destrucción de eritrocitos (angiosarcomas, granuloma piógeno y tejido de granulación inespecífico), son útiles para reconocer al SK cuando se usan en combinación con otros criterios histológicos (Furunaga 1991).

Normalmente la epidermis y los anejos cutáneos suelen permanecer intactos en el SK. Los cambios epidérmicos observados varían con el estadio de la lesión, observándose en las fases más avanzadas atrofia y ulceración epidérmica, sobre todo en las lesiones más sobreelevadas. Ocasionalmente puede apreciarse un collarete epidérmico en los límites de las pápulas y de los nódulos.

Se ha cuestionado largamente si los hallazgos histológicos del SK poseen trascendencia pronóstica. Parece razonable pensar que si la enfermedad progresa de mácula a placa y de placa a tumor, la presencia de características histológicas específicas de cada uno de los distintos estadios podría ser una guía de la evolución clínica. De todas formas, el estrecho solapamiento histológico antes comentado entre los diferentes estadios del SK hace poco probable esta posibilidad (Elder 1997; Weedon 1997).

4.6. VARIANTES HISTOLÓGICAS DEL SK

Como se ha comentado previamente, en general las lesiones típicas del SK no muestran pleomorfismo y su índice mitótico es bajo. No obstante, se han descrito formas histológicamente agresivas o anaplásicas, con un carácter obviamente sarcomatoso, y que probablemente son el resultado de una desdiferenciación histológica progresiva de casos que inicialmente eran típicos (Weiss 2001). De todos modos, también se han descrito tumores pobremente diferenciados desde su inicio, lo que parece ser más frecuente en los casos de SK originados en África (Taylor 1971; O'Connell 1977). Estas lesiones anaplásicas exhiben una mayor celularidad, un mayor grado de atipia citológica en lo que respecta al tamaño, forma y características nucleares, y un incremento de la actividad mitótica. Asimismo puede observarse una reducción o incluso pérdida del componente vascular. Por todo ello, estas formas histológicas del SK puede tener áreas muy similares a las del angiosarcoma o a las del fibrosarcoma.

En raras ocasiones las lesiones del SK pueden ofrecer una apariencia histológica linfangiomatosa, caracterizada por la presencia de luces vasculares groseras e irregulares revestidas por una única capa de células endoteliales aplanadas y no acompañadas por eritrocitos intravasculares, ni extravasados en la dermis. La ausencia de depósitos de hemosiderina y la escasez de células fusiformes contribuyen aún más a este aspecto linfangiomatoso de las lesiones. Este patrón suele observarse focalmente en el contexto de una lesión de SK típica, generalmente en la periferia de los tumores sólidos. No obstante, en biopsias de pacientes con múltiples lesiones se ha observado que algunas de las muestras están constituidas completamente por este patrón pseudolinfangiomatoso,

mientras que otras muestras del mismo paciente ofrecen un patrón típico del SK. Los casos de SK en los que predominan estos canales tipo linfangioma se denominan en la literatura como SK pseudolinfangiomatoso (Gange 1979; Cossu 1997).

Debe tenerse en cuenta que algunas lesiones vasculares de la piel de pacientes con SIDA no muestran las características morfológicas típicas del SK. Pueden observarse grandes canales vasculares anastomosados similares a los del hemangioma o linfangioma, que adicionalmente muestran protusiones papilares revestidas por células endoteliales atípicas emulando el aspecto histológico del angiosarcoma; otros están compuestos por células endoteliales histiocitoides o epitelioides; algunos son sólidos e indiferenciados; e incluso se han identificado apariencias pseudolinfangiomatosas como las descritas en el SK no asociado a SIDA (Weiss 2001).

Frecuentemente, el estadio nodular se acompaña por la afectación de órganos internos, sobre todo ganglios linfáticos y vísceras, y esto ocurre predominantemente en los pacientes africanos y en los infectados con el VIH-1. De igual forma que los cambios iniciales de SK cutáneo son un reto diagnóstico, lo mismo ocurre en las lesiones iniciales en otros órganos, sobre todo en los ganglios linfáticos. Los cambios incipientes en los ganglios linfáticos pueden estar representados por una moderada angiectasia y proliferación vascular en los senos subcapsulares, expandiéndose hasta afectar gradualmente a los sinusoides interfoliculares. Estos cambios son muy similares a varias condiciones reactivas nodales como la angiomatosis nodal y la transformación vascular de los senos subcapsulares. Otros han denotado la similitud de estos ganglios linfáticos con la enfermedad de Castleman, sobre todo cuando los vasos proliferantes están centrados alrededor de los folículos

(Frizzera 1983). Para realizar un diagnóstico correcto de estos ganglios linfáticos histológicamente ambiguos, debe incluirse la totalidad del tejido biopsiado, lo que generalmente permite identificar la presencia de focos fusocelulares más sólidos que confirman el diagnóstico de SK ganglionar (O'Connell 1977).

Los casos avanzados de afectación nodal por SK no presentan tanta problemática, ya que normalmente muestran una sustitución parcial o completa del parénquima linfático por una proliferación fusocelular monótona. Debido a que los pacientes con SIDA son susceptibles de desarrollar pseudotumores secundarios a micobacterias en los ganglios linfáticos, así como cambios asociados a diversas infecciones, deben realizarse técnicas especiales para distinguir estos cambios del SK nodal. Ocasionalmente el SK nodal coexiste con linfomas malignos o leucemias (Weshler 1979).

5. DIAGNÓSTICO DIFERENCIAL HISTOLÓGICO

El diagnóstico de un caso de SK bien establecido clínica e histopatológicamente ofrece pocas dificultades. Los principales errores diagnósticos se originan cuando se interpretan las lesiones de estadios iniciales como una inflamación banal o bien como alguna anomalía linfática o angiomatosa menor. La histopatología de las lesiones nodulares completamente desarrolladas del SK es en general distintiva y raramente plantea problemas diagnósticos. No obstante, en las lesiones muy avanzadas o en los casos agresivos, donde la atipia citológica se incrementa, el componente vascular es escaso y la proliferación fusocelular es extensa y prominente, el diagnóstico diferencial con lesiones malignas es complejo (Elder 1997; Requena 1997; Weedon 1997; Requena 1998; Weiss 2001).

5.1. DIAGNÓSTICO DIFERENCIAL EN LOS ESTADIOS INICIALES

Las lesiones iniciales del SK son sutiles microscópicamente y deben diferenciarse de condiciones inflamatorias reactivas y de proliferaciones o tumores vasculares benignos. La dificultad se incrementa especialmente en los pacientes VIH positivos, ya que como se ha comentado previamente, reconocer los cambios incipientes del SK-SIDA es un problema diagnóstico difícil de solucionar (Weiss 2001).

Las lesiones maculares iniciales en las que es poco evidente la naturaleza vascular de la lesión, pueden confundirse con un histiocitoma fibroso benigno pobremente celular o atrófico (Blumenfeld 1985). Los marcadores inmunohistoquímicos apropiados pueden clarificar la naturaleza vascular de la lesión subyacente (CD31 y/o CD34) o poner de relieve la lesión histiocitaria (Factor XIIIa).

Normalmente una elevada presión venosa, con la consiguiente hipostasis, puede ser secundaria a una insuficiencia o incompetencia venosa profunda y, aunque menos comúnmente, a anomalías arteriovenosas adquiridas o congénitas. Esta patología es responsable de la aparición de una serie de lesiones cutáneas angioproliferativas, denominadas pseudokaposiformes por su parecido con el SK. Entre ellas se encuentran la acroangiodermatitis o pseudosarcoma de Kaposi, las dermatitis de éstasis y las malformaciones arteriovenosas (Blumenfeld 1985; Chor 1992). El diagnóstico diferencial histológico del SK con las lesiones pseudokaposiformes es en general sencillo.

Histológicamente, las lesiones pseudokaposiformes afectan a los vasos sanguíneos de la dermis papilar, observándose una expansión de todo el lecho vascular dérmico a expensas de una reduplicación y marcada dilatación de los capilares, que además muestran una morfología en sacacorchos y, en general, una apariencia angiomatosa. De forma similar, las vénulas y pequeñas venas profundas verticales se hipertrofian volviéndose tortuosas. Los capilares son redondeados, poseen una pared gruesa y se encuentran revestidos por células endoteliales prominentes. Estos capilares angiomatosos están separados entre sí por una matriz edematosa, no existiendo el fenómeno de "back-to-back" tan típico del SK. Otros hallazgos adicionales que permiten el diagnóstico diferencial son la presencia de un fondo de fibrosis dérmica con células fusiformes orientadas horizontalmente, eritrocitos extravasados y numerosos hemosiderófagos (Strutton 1987).

Al contrario que los vasos redondos y uniformes de estas lesiones pseudokaposiformes, los vasos del estadio inicial del SK son irregulares y angulados, delimitando luces en hendidura y asociándose frecuentemente a un infiltrado inflamatorio mononuclear con numerosas células plasmáticas. Adicionalmente, los vasos proliferantes del SK también se extienden rodeando capilares preexistentes y afectando la adventicia perianexial de los anejos dérmicos. Una clave diferencial entre el SK y el pseudosarcoma de Kaposi es que en este último los fenómenos de hiperplasia afectan a la vasculatura preexistente, mientras que en el primero existe una proliferación vascular propiamente dicha. De todas formas, hay que tener en cuenta que las enfermedades vasculares hipostáticas pueden coexistir con el SK.

Ocasionalmente, las lesiones cutáneas secundarias a malformaciones arteriovenosas pueden duplicar clínicamente el aspecto del SK (Marshall 1985). Histológicamente, estas lesiones consisten en una proliferación de pequeños vasos de calibre capilar en algunos casos rodeados por eritrocitos extravasados y hemosiderina. No se aprecia la formación de hendiduras luminales, ni tampoco una morfología celular fusiforme evidente. La realización de estudios arteriográficos documenta la presencia de una malformación arteriovenosa subyacente.

Los vasos del SK son mucho más irregulares que los vasos de las telangiectasias adquiridas o de las dermatosis purpúricas pigmentadas. Asimismo, estas entidades raramente presentan un infiltrado inflamatorio que incluya células plasmáticas, frecuente en las lesiones iniciales del SK.

Cuando los cambios inflamatorios predominan, el componente vascular puede pasar fácilmente desapercibido y entonces el diagnóstico diferencial incluye a las dermatosis inflamatorias superficiales. Excepcionalmente se encuentran células plasmáticas en las dermatosis inflamatorias superficiales; sin embargo, éstas son muy abundantes en la sífilis secundaria, debiéndose considerar esta posibilidad diagnóstica. No obstante, y al contrario que en el SK, donde los contornos vasculares son irregulares y están revestidos por un endotelio relativamente aplanado, las estructuras vasculares presentes en la sífilis secundaria muestran una marcada obliteración luminal a expensas de una hiperplasia y edema de las células endoteliales.

En algunas localizaciones, las lesiones del SK pueden infiltrar los haces colágenos de forma insidiosa, asemejándose a la infiltración por histiocitos que se aprecia en las lesiones incipientes de los granulomas anulares o de la necrobiosis lipoídica. De todas formas, en el SK estas células infiltrantes rodean a vasos preexistentes, un hallazgo que no se observa en los granulomas en empalizada.

Otras condiciones vasculares adquiridas, como los hemangiomas capilares, la angiomatosis bacilar, el granuloma piógeno y el tejido de granulación de las cicatrices en formación también pueden causar problemas diagnósticos desde un punto de vista clínico-patológico. Los dos últimos suelen confundirse con las lesiones del estadio de placa del SK y ocasionalmente con las lesiones nodulares, ya que éstas pueden ulcerarse desarrollando un componente superficial reactivo parecido al del tejido de granulación o al del granuloma piógeno. De nuevo, reconocer los espacios vasculares angulados que se forman en el SK en comparación con los vasos regulares y más redondeados de los tumores vasculares benignos y proliferaciones reactivas permite su distinción.

En las lesiones maculares tardías, el diagnóstico diferencial incluye el hemangioma hemosiderótico en diana, el linfangioendotelioma benigno, el hemangioma microvenular y el angiosarcoma bien diferenciado.

El hemangioma hemosiderótico en diana comparte también algunas características histopatológicas con el SK. En las áreas periféricas del halo hemosiderótico, las luces vasculares de morfología angulada e irregular, que parecen estar disecando los haces colágenos dérmicos, sugieren una lesión inicial del SK. Al contrario que en el SK

temprano, las células inflamatorias son escasas y el depósito de hemosiderina es abundante. Sin embargo, en las áreas centrales del hemangioma targetoide hemosiderótico encontramos luces vasculares ampliamente dilatadas con proyecciones papilares intraluminales, células endoteliales prominentes en la luz vascular a modo de tachuela o "hobnail" y frecuentes trombos de fibrina, hallazgos no identificables en el SK y que facilitan el diagnóstico diferencial.

Histológicamente el linfangioendotelioma benigno es difícilmente distinguible del SK con patrón pseudolinfangiomatoso. Hay numerosas similitudes entre ambas entidades, como la presencia de espacios vasculares de paredes finas, revestidos por células endoteliales aplanadas y localizados entre los haces colágenos disecando la dermis. Sin embargo, los espacios vasculares del linfangioendotelioma benigno se distribuyen generalmente de forma horizontal en la dermis y no muestran la tendencia a rodear estructuras dérmicas preexistentes como en el SK. Además, la ausencia de eritrocitos extravasados, hemosiderina y células plasmáticas son también características que ayudan en el diagnóstico diferencial. Lo mismo ocurre con los datos clínicos, dado que el linfangioendotelioma benigno es una lesión única.

El hemangioma microvenular está constituido por vasos que contienen hematíes, muchos de los cuales están rodeados por pericitos o células musculares lisas -positivas para la tinción inmunohistoquímica con Actina- sugestivas de diferenciación perivenular.

Hallazgos histológicos típicos del SK como son los vasos irregulares, la disección de los haces colágenos por estructuras vasculares y el infiltrado inflamatorio crónico pueden

verse también en el angiosarcoma de bajo grado o bien diferenciado. La evidencia de células endoteliales que protuyen en la luz vascular y que muestran una marcada atipia celular permiten la distinción en la mayoría de los casos, ya que el SK se caracteriza por un endotelio más aplanado y de apariencia banal. Aún así, el diagnóstico diferencial entre el angiosarcoma y el SK puede ser muy difícil en algunos casos, dado que cada una de estas lesiones puede contener focos de diferenciación que similares a la otra. En estos casos es necesario efectuar un muestreo extenso y realizar múltiples niveles de corte de los bloques tisulares para establecer un diagnóstico correcto.

5.2. DIAGNÓSTICO DIFERENCIAL EN LOS ESTADIOS AVANZADOS

Las lesiones del SK con componente fusocelular deben diferenciarse de las siguientes entidades: tumores cutáneos de músculo liso, histiocitoma fibroso aneurismático, hemangioendotelioma kaposiforme de los adolescentes, hemangioendotelioma de células fusiformes, angiohistiocitoma de células gigantes y angiosarcoma con diferenciación fusocelular. Estas lesiones son las simuladoras histológicas más importantes de las lesiones nodulares del SK

Los tumores cutáneos de músculo liso no muestran la estrecha relación típica de células fusiformes, hendiduras vasculares y glóbulos hialinos. Además, las células fusiformes muestran generalmente positividad inmunohistoquímica para filamentos intermedios de desmina.

El histiocitoma fibroso benigno aneurismático es una lesión fusiforme altamente celular y no vascular, pero con presencia de espacios pseudovasculares y depósito de hemosiderina, que histológicamente puede confundirse con el estadio nodular del SK (Calonge 1995). La presencia de áreas periféricas similares al histiocitoma fibroso dérmico habitual, histiocitos espumosos, células gigantes multinucleadas, cambios epidérmicos típicos asociados al histiocitoma y, finalmente, el perfil inmunohistoquímico, evitarían un diagnóstico erróneo.

El hemangioendotelioma kaposiforme comparte un llamativo parecido con las lesiones nodulares del SK, aunque los contextos clínicos son muy distintos (Zukerberg 1993; Lam 1995). El hemangioendotelioma kaposiforme se presenta principalmente en niños y es una

lesión solitaria que generalmente afecta a las partes blandas profundas, y secundariamente a la piel. A nivel histológico el tumor muestra características intermedias entre el hemangioma capilar y el SK. No obstante, posee un patrón de crecimiento lobulado con áreas pseudohemangiomatosas en la periferia de los lóbulos que está ausente en el SK.

El hemangioendotelioma de células fusiformes es una entidad que se confunde frecuentemente a nivel histológico con la fase nodular o tumoral del SK, ya que en ambas entidades hay fascículos de células fusiformes con formación de hendiduras vasculares que contienen eritrocitos. La detección de espacios vasculares cavernosos ampliamente dilatados y colecciones de células endoteliales epitelioides con formación de luces intracitoplasmáticas, que no se observan en el SK, son los hallazgos más fiables para distinguir ambos tumores (Weiss 2001).

El angiohistiocitoma de células gigantes es una entidad vascular reactiva descrita en mujeres de edad avanzada y que debe considerarse también en el diagnóstico diferencial del SK. Clínicamente se origina como unas pápulas vasculares agrupadas que crecen lentamente, y suelen localizarse en las piernas, aunque también se han descrito en la cara y en las manos. Histológicamente, se identifica un incremento del número de capilares y vénulas dérmicas, que ocasionalmente pueden dilatarse hasta alcanzar proporciones angiomatosas. La lesión se acompaña de escasas células inflamatorias y ocasionales histiocitos y células multinucleadas dispersos en la dermis. El depósito de pigmento sanguíneo suele ser escaso (Smolle 1989; Jones 1990).

Adicionalmente hay que descartar la existencia de lesiones de células fusiformes benignas que han sufrido algún traumatismo, como por ejemplo el leiomioma o el dermatofibroma. Debido al trauma las células fusiformes propias de estas lesiones parecen formar espacios en hendidura que contienen eritrocitos. La ausencia de hemosiderina y de actividad mitótica, y las técnicas inmunohistoquímicas pueden ser útiles para excluir el SK. Sin embargo, es necesaria una extirpación completa de la lesión para poder identificar las regiones no traumatizadas, lo que permitirá realizar una evaluación correcta de su naturaleza.

Un pequeño número de angiosarcomas cutáneos, principalmente de alto grado, muestran diferenciación fusocelular. Suelen estar compuestos por escasos espacios vasculares y una proliferación densa de células de fenotipo fusiforme, aunque pleomórficas y con una elevada actividad mitótica. Focalmente, estos tumores pueden tener áreas bien diferenciadas que faciliten su diagnóstico. Generalmente son tumores pobremente diferenciados de difícil distinción, con carcinomas o fibrosarcomas de alto grado, por lo que el diagnóstico diferencial con el estadio tumoral del SK no suele ser complicado.

Finalmente, en el diagnóstico diferencial de las lesiones agresivas tardías deben considerarse los tumores malignos de células fusiformes. Los más importantes a tener en cuenta son el carcinoma escamoso de células fusiformes, el fibrosarcoma, el leiomiosarcoma, el sarcoma sinovial monofásico, el nevus azul celular maligno con escaso depósito de melanina y el melanoma maligno desmoplásico. Generalmente estos tumores son más anaplásicos que el SK y muestran un perfil inmunohistoquímico característico que

permite el diagnóstico en la mayoría de los casos. De todas formas, una precisa historia clínica es de suma importancia para establecer un diagnóstico correcto.

5.3. RESUMEN DE LOS DIAGNÓSTICOS DIFERENCIALES DEL SK

- **EN LOS ESTADIOS INICIALES:**
    - Histiocitoma fibroso benigno pobremente celular o atrófico
    - Acroangiodermatitis o pseudosarcoma de Kaposi/dermatitis de éstasis
    - Malformaciones arteriovenosas
    - Telangiectasias adquiridas
    - Dermatosis purpúricas pigmentadas
    - Dermatosis inflamatorias superficiales (sífilis secundaria)
    - Granulomas anulares/necobiosis lipoídica
    - Hemangiomas capilares
    - Angiomatosis bacilar
    - Granuloma piógeno
    - Tejido de granulación de las cicatrices en formación
    - Hemangioma hemosiderótico en diana
    - Linfangioendotelioma benigno
    - Hemangioma microvenular
    - Angiosarcoma de bajo grado o bien diferenciado

- **EN LOS ESTADIOS AVANZADOS:**
    - Tumores cutáneos de músculo liso
    - Histiocitoma fibroso benigno aneurismático
    - Hemangioendotelioma kaposiforme
    - Hemangioendotelioma de células fusiformes
    - Angiohistiocitoma de células gigantes
    - Lesiones de células fusiformes benignas que han sufrido algún traumatismo (leiomioma, dermatofibroma)
    - Angiosarcoma con diferenciación fusocelular
    - Tumores malignos de células fusiformes:
        - carcinoma escamoso de células fusiformes
        - fibrosarcoma
        - leiomiosarcoma
        - sarcoma sinovial monofásico
        - nevus azul celular maligno con escaso depósito de melanina
        - melanoma maligno desmoplásico

6. HISTOGÉNESIS: ORIGEN DE LAS CÉLULAS DEL SK

Todavía existe una cierta incertidumbre respecto al origen histogénico de las células que proliferan en el SK. En particular, la histogénesis del componente fusocelular de las lesiones de placa y nodulares del SK ha sido objeto de una considerable controversia, y aún hoy es un tema polémico.

El establecimiento de cultivos de células fusiformes a partir de células mononucleares de la sangre periférica de pacientes con SK-SIDA ha permitido examinar *in vitro* las propiedades inmunofenotípicas y características funcionales de las células del SK, observándose que coinciden con las de las células endoteliales de la microvasculatura dérmica. Estos hallazgos confirman la principal localización de la célula endotelial a partir de la cual se originan las células del SK en la mayoría de los casos. Estudios de varios grupos han mostrado que el SK está constituido por una proliferación celular mesenquimal de evidente diferenciación vascular (Nadji 1981). Sin embargo, los resultados son aparentemente contradictorios respecto a si estas células representan endotelio de vasos sanguíneos o de vasos linfáticos ya variabilidad de los diversos resultados observados en la literatura no hace más que reflejar esta problemática.

Desde un punto de vista histogénico, los estudios inmunohistoquímicos y ultraestructurales han contribuido de forma significativa a la compresión del origen de las células que componen el SK.

6.1. INMUNOHISTOQUÍMICA

Las células que revisten las estructuras vasculares bien diferenciadas de las lesiones iniciales del SK contienen el antígeno relacionado con el factor-VIII. No obstante, estudios de este marcador en las células fusiformes tumorales han mostrado resultados contradictorios, originando una controversia respecto a la naturaleza endotelial del SK. Este factor parece tener una expresión muy variable en los tumores vasculares y, por lo tanto, está sujeto a una gran variedad de interpretaciones, probablemente secundarias, a los diferentes procesos y reactivos inmunohistoquímicos utilizados (Weiss 2001). La posterior utilización de un mayor número de anticuerpos dirigidos contra diferentes antígenos estructurales de las células endoteliales ha permitido subsanar estos problemas (Millard 1985). En contraste, la lecitina de *Ulex europaeus-I* está presente de forma constante en las células del SK (Jones 1986).

Mediante la positividad para OKM5, anti-E92 y HC1, tres anticuerpos monoclonales que reaccionan con el endotelio vascular pero no con el linfático, Rutgers et al. afirman que las células fusiformes del SK son células endoteliales de origen vascular sanguíneo.

Scully et al. llegaron a las mismas conclusiones basándose en la inmunoreactividad de las células fusiformes para el anticuerpo B721, que reacciona con todos los endotelios vasculares excepto con el de los glomérulos renales y el de los sinusoides hepáticos y esplénicos.

La presencia de abundante laminina y de colágeno tipo IV rodeando a muchas de las células fusiformes del SK ha sido interpretada como una evidencia histoquímica que favorece la diferenciación endotelial vascular frente a la endotelial linfática (Bendelac 1985; Kramer 1985; Penney 1988).

Asimismo, diferentes investigadores han demostrado que las células del SK expresan intensamente CD34, también conocido como el antígeno de células progenitoras humanas. Esta molécula es una glicoproteína transmembrana de cadena simple de 105 a 120 kDa, expresada constitutivamente por las células endoteliales de los pequeños vasos sanguíneos de diversos tejidos, pero no por las células de los vasos linfáticos (Kraffert 1991; Nickoloff 1991). De todas formas los resultados obtenidos respecto al CD34 son variables según los estudios.

En otros estudios, las células del SK parecen reaccionar con anticuerpos dirigidos contra antígenos comunes presentes tanto en las células endoteliales sanguíneas como en las linfáticas, como el *Ulex europaeus-I* y el EN-4, pero no reaccionan con ciertos marcadores específicos de células endoteliales sanguíneas como el factor de von Willebrand (antígeno relacionado con el factor VIII) y el anticuerpo PAL-E. Por lo tanto, esto favorece la hipótesis de que las células de SK derivan probablemente de las células endoteliales linfáticas o bien siguen una diferenciación linfática (Fitzpatrick 1999).

Beckstead, Wood y Fletcher también favorecen la hipótesis de una diferenciación endotelial linfática por la ausencia de HLA-DR/Ia y fosfatasa alcalina, y por la intensa tinción con 5'-nucleotidasa. Posteriormente, un marcador relativamente sensible de

diferenciación linfática, el receptor del factor de crecimiento endotelial vascular-3 (VEGFR-3), ha sido identificado en la mayoría de los SK (Jussila 1998; Weninger 1999; Folpe 2000).

Jones et al. han observado que la inmunoreactividad del SK varía con el estadio de la enfermedad y el tipo de las lesiones. En los estadios iniciales el perfil inmunohistoquímico es el de un tumor linfático, de forma que las células tumorales se tiñen positivamente con un anticuerpo dirigido contra cualquier endotelio (EN-4), pero no se tiñen con un anticuerpo específico para el endotelio vascular sanguíneo (PAL-E). Sin embargo, las lesiones más desarrolladas (nodulares) se tiñen con el EN-4 y expresan una inmunoreactividad variable con PAL-E, mostrando una naturaleza mixta.

Comparando los resultados inmunohistoquímicos anteriores con los hallazgos histológicos de las fases iniciales -aspecto pseudolinfangiomatoso y formación de pequeños canales veno-linfáticos-, algunos autores opinan que la lesión podría originarse a partir de células endoteliales linfáticas más que de células endoteliales vasculares. No obstante, teniendo en cuenta el perfil inmunohistoquímico del SK en las diferentes fases de la enfermedad y la estrecha relación histogénica entre venas y linfáticos, otros autores opinan que un endotelio con características híbridas puede ser el responsable de los aspectos sanguíneos y linfáticos que se aprecian en el SK (Dictor 1988).

Finalmente, algunos autores han postulado un origen no vascular para las células fusiformes del SK. Se ha sugerido que el dendrocito dérmico, un miembro del sistema fagocítico mononuclear que expresa el factor XIIIa, puede ser la célula de origen de las

células fusiformes del SK (Nickoloff 1989; Huang 1993). Se han identificado numerosas células positivas para el factor XIIIa y otros antígenos asociados a células dendríticas/macrofágicas como el CD68 y el receptor de la manosa en las lesiones del SK. No obstante, los resultados inmunohistoquímicos respecto a la positividad del factor XIIIa han sido muy variables en los diferentes estudios, por lo que es poco probable que las células fusiformes deriven de los dendrocitos dérmicos.

Algunos autores creen que estas células dendrocíticas positivas para el factor XIIIa, presentes en mayor o menor cantidad en las lesiones del SK, representan una hiperplasia reactiva, más que propiamente células neoplásicas (Gray 1991). De todas formas su presencia sugiere que los dendrocitos activados pueden tener un papel importante en la iniciación y proliferación de las células fusiformes en las lesiones del SK.

Más recientemente, Weich et al. han propuesto que las células del SK pueden estar estrechamente relacionadas con las células musculares lisas vasculares, ya que las lesiones tumorales expresan ARNm de α-actina de músculo liso.

6.2. ULTRAESTRUCTURA

Tradicionalmente el microscopio electrónico ha apoyado la idea de una diferenciación endotelial genérica del SK. Los estudios han documentado que la mayoría de las células fusiformes del SK muestran características ultraestructurales de células endoteliales, sobre todo en las fases más iniciales. Sin embargo, algunas de ellas también poseen características de pericitos y fibroblastos (Ruszczak 1987).

En las lesiones iniciales se aprecia como las células endoteliales que revisten las hendiduras luminales son delgadas y finas, y muestran un núcleo ovalado y un nucléolo pequeño. Estas células presentan escasas uniones intercelulares, identificándose focalmente uniones tipo gap entre células adyacentes. La superficie externa de las células luminales se encuentra rodeada por lámina basal fragmentada, y ocasionalmente se identifican pericitos.

Las lesiones más avanzadas contienen células que han sido descritas como “periteliales” o “fibroblásticas”, ya que ultraestructuralmente poseen lisosomas y ferritina y, por lo tanto, poseen actividad fagocítica. De todas formas, observaciones inmunohistoquímicas simultáneas indican que en realidad se tratan de células endoteliales modificadas (Weiss 2001).

Actualmente, los estudios ultraestructurales han mostrado similitudes morfológicas significativas entre las células del SK y el endotelio de los capilares linfáticos dérmicos. Es decir, las características ultraestructurales parecen ser más compatibles con diferenciación vascular linfática que con diferenciación vascular sanguínea (Requena 1998). Aún así, se

ha barajado la posibilidad de que la ausencia de características propiamente sanguíneas sea el resultado de alteraciones de tipo telangiectásicas en los vasos del SK (McNutt 1983).

6.3. CONCLUSIÓN

En resumen, las actuales evidencias inmunohistoquímicas y ultraestructurales sugieren que la histogénesis de las células del SK está estrechamente relacionada con las células endoteliales linfáticas. No obstante, no se ha podido descartar completamente que el progenitor de las células del SK sea una célula precursora endotelial indiferenciada circulante.

7. NATURALEZA DEL SK

La naturaleza del SK es incierta y no está todavía completamente definida. Esta incertidumbre se refleja claramente cuando se observa la clasificación histológica del SK en los diferentes libros especializados. Aunque todos los autores coinciden en localizarlo dentro de los tumores de partes blandas de estirpe vascular, su distribución en los distintos grupos de comportamiento es mucho más variable, ya que no se ha aclarado todavía si debe incluirse en el grupo de neoplasias malignas o en el de tumores de comportamiento incierto. Weiss, Requena y la O.M.S. lo clasifican dentro de las neoplasias malignas junto con los angiosarcomas. Por otro lado, Robbins, Lever y Weedon los incluyen en el grupo de tumores de grado intermedio, comportamiento incierto o malignos de bajo grado, junto con el hemangioendotelioma y el hemangiopericitoma.

Ya desde su descripción se han sucedido diferentes opiniones sobre la naturaleza de esta lesión. La diferente evolución clínica según la variante que se padezca, la existencia de casos agresivos frente a otros evidentemente benignos y la regresión espontánea en algunos casos (coincidiendo con la recuperación de la respuesta inmune normal) o en asociación con el embarazo (probablemente como resultado del efecto modulatorio de la gonadotropina coriónica humana en la proliferación vascular) (Lunardi-Iskandar 1995; Pfeffer 2002) desorientan tanto a clínicos como a patólogos e investigadores.

La evolución lenta e indolente (especialmente en la forma clásica de la enfermedad), la regresión espontánea en algunos casos y la escasa atipia histológica, hace que algunos autores consideren al SK, al menos inicialmente, como un proceso angioproliferativo

reactivo multifocal. Asimismo, estudios animales que han utilizado células inmortalizadas de SK-SIDA sugieren que el SK es un proceso reactivo con un comportamiento incierto y no una neoplasia. La multifocalidad y la existencia de formas malignas o anaplásicas con una supervivencia corta hacen que otros autores opinen que el SK es un verdadero sarcoma con potencial metastásico. Es decir, algunos autores consideran al SK como un proceso angioproliferativo reactivo multifocal, mientras que otros opinan que se trata de un verdadero sarcoma aunque con un bajo potencial metastático.

Opiniones intermedias definen al SK como una entidad evolutiva que comienza en sus fases iniciales como una proliferación vascular reactiva benigna causada por una red desequilibrada de citocinas, y acaba evolucionando hasta convertirse, en los estadios avanzados, en una verdadera neoplasia multifocal, ya que se ha demostrado en algunas de las lesiones multicéntricas un origen monoclonal (Rabkin 1997). De hecho, esta naturaleza binomial (hiperplásica/neoplásica) del SK es una de las características más fascinantes de esta enfermedad.

En las biopsias de piel clínicamente no afecta de pacientes con SK y SIDA se han observado cambios vasculares atípicos incipientes (Schwartz 1984; De Dobbeleer 1987). Esto sugiere la opción de que en el contexto de esta inmunodeficiencia se desarrollan alteraciones endoteliales generalizadas en el organismo del paciente, y que algunas de ellas podrían evolucionar a lesiones clínicamente evidentes. Teniendo esto en cuenta, la afectación diseminada del SK no representaría propiamente un proceso de extensión metastásico, sino una afectación multifocal (Dorfman 1986).

Como hemos visto previamente en el apartado de histogénesis, las lesiones del SK contienen células fusiformes que comparten características con las células endoteliales, sobre todo linfáticas, y también con las células musculares lisas, por lo que es probable que el SK se origine de células mesenquimales primitivas capacitadas para formar canales vasculares. Algunos estudios parecen mostrar que estas células son de origen monoclonal (Rabkin 1997), incluso en los pacientes con lesiones multicéntricas, lo que indicaría por tanto que el SK es una neoplasia. No obstante, los estudios que han analizado la clonalidad de las lesiones del SK mediante patrones de inactivación del cromosoma X han mostrado que también pueden ser policlonales (Gill 1998). Asimismo, la presencia de telomerasas activas en el SK (Chen 2001) sugiere una naturaleza neoplásica del SK.

Actualmente la opinión intermedia es la más extendida. Así, en sus estadios iniciales el SK no se considera una verdadera neoplasia, sino que se trata de un proceso angioproliferativo multifocal. Mientras que en las lesiones avanzadas que muestran algunas características de progresión tumoral (crecimiento nodular expansivo, células atípicas y un curso biológico más agresivo), es probable que representen una lesión neoplásica sin potencial metastásico.

Ante la presencia de resultados contradictorios y dada la ausencia de datos fidedignos, resulta evidente la necesidad de realizar estudios genotípicos que definan los diferentes estadios evolutivos del SK, ya que sus resultados serían de una gran relevancia para determinar con exactitud su comportamiento, evolución y, en última instancia, el tratamiento más acertado del SK en cada caso.

8. EVOLUCIÓN Y CURSO CLÍNICO

El comportamiento biológico del SK es variable y depende de un gran número de factores interrelacionados: el nivel de inmunocompetencia del huésped, el tipo epidemiológico, el estadio de la enfermedad, y la presencia o ausencia de infecciones oportunistas.

Aunque se desconoce si el SK representa una proliferación vascular reactiva o una verdadera neoplasia, actualmente hay un acuerdo general en que el SK no produce metástasis de la misma forma que los sarcomas convencionales, sino que más bien se desarrolla de modo multifocal (ver apartado 7). Aún así, pese a no comportarse como un verdadero sarcoma, los pacientes pueden fallecer por los efectos del SK.

En general, la afectación de la forma clásica está restringida a la superficie cutánea, mientras que en las formas endémica y epidémica la afectación suele ser más extensa y localizarse con más frecuencia en órganos internos. Consecuentemente, la variante clásica es relativamente indolente y compatible con una supervivencia larga, y los tipos endémico y epidémico suelen ser más agresivos.

Los individuos con la forma clásica del SK se presentan con una enfermedad cutánea limitada que progresa lentamente y con rara afectación visceral o de los ganglios linfáticos. En los pacientes de este grupo la mortalidad en relación con la enfermedad es de entre el 10% y el 20%, durante un período de seguimiento de 10 años, y la duración de la enfermedad es de entre 8 a 10 años (Requena 1998). En general, la muerte suele ocurrir

años después, a causa de enfermedades intercurrentes no relacionadas, aunque el 25% de los pacientes fallecen de un tumor maligno secundario, particularmente de tipo linfoide.

La afectación precoz de los ganglios linfáticos y la extensa infiltración de las partes blandas y órganos internos hace que el SK africano sea mucho más agresivo que el SK convencional. Frecuentemente estos pacientes fallecen uno o dos años después del diagnóstico de la enfermedad (Odom 2000).

El SK en pacientes con SIDA posee un curso mucho más agresivo. La media de mortalidad en los pacientes con SK-SIDA es del 41%, durante un período de seguimiento relativamente corto (Mitsuyasu 1987; Requena 1998). Esta mortalidad está marcadamente influenciada por el estadio de la enfermedad y por la presencia de infecciones oportunistas y síntomas sistémicos. De hecho, el SK asociado a SIDA es extenso pero casi nunca fatal, y en un período de 28 meses, el 80% de los pacientes que no han sufrido infecciones oportunistas están vivos, mientras que menos del 20% de los que han sufrido una infección oportunista sobreviven (Krigel 1984).

El curso de la enfermedad es variable en pacientes que desarrollan SK por inmunosupresión yatrogénica. En muchos casos la supresión de los fármacos puede representar la resolución del SK sin terapia asociada.

9. TRATAMIENTO

La selección apropiada de la terapia en cada caso depende de la variante clínico-epidemiológica de la enfermedad, de la extensión y la localización de las lesiones, y de la competencia inmunológica del paciente.

Las opciones terapéuticas en los diferentes contextos clínicos del SK incluyen las terapias locales y/o sistémicas. La combinación de varias terapias utilizando radiación, quimioterapia e inmunoterapia parece ser el tratamiento de elección en SK (Tappero 1993; Requena 1998).

Las terapias locales incluyen escisión quirúrgica, crioterapia con nitrógeno líquido, ablación con láser, terapia con radiación ionizante y terapia intradérmica con fármacos quimioterápicos citotóxicos o IFN-α.

La terapia sistémica es necesaria cuando se desarrollan más de 10 nuevas lesiones de SK en menos de 1 mes, si hay linfedema asociado o si existe afectación visceral sintomática. En estas terapias se suele emplear el IFN-α, con o sin zidovudina asociada, y en intervenciones más agresivas, la combinación de varios agentes quimioterápicos (Mauss 1995). La tendencia a la multifocalidad hace que la quimioterapia y la irradiación sean en muchos casos terapias de elección.

Todas las variantes del SK son radiosensibles y por ello la terapia con radiación ionizante ha sido utilizada con un considerable éxito y actualmente es una de las opciones más extendidas (Odom 2000).

Algunos pacientes con enfermedad rápidamente progresiva requieren quimioterapia con fármacos citotóxicos. La combinación de drogas más comúnmente empleada utiliza la doxorrubicina, bleomicina y vincristina. A pesar de su toxicidad, estas combinaciones son razonablemente seguras y ciertamente muy efectivas en el tratamiento paliativo de los casos avanzados de SK. Incluso se han descrito algunos casos de remisión completa (Coukell 1997).

En algunos estudios se ha identificado la inhibición del crecimiento de ciertos tumores inducidos por virus -carcinoma nasofaríngeo, papilomas laringeos y genitales- tras la aplicación de interferones humanos (naturales o recombinantes). Esto ha originado varios experimentos terapéuticos con IFN-α en el SK debido a su asociación con el VHH-8. El IFN-α se ha utilizado solo, combinado con terapia citotóxica, o conjuntamente con zidovudina en los pacientes con SIDA (Mauss 1995). Aunque los resultados de estos estudios son contradictorios, muestran que la eficacia de la terapia con IFN-α depende, al menos en parte, de un sistema inmune intacto.

Actualmente se ha demostrado que el tratamiento con agentes anti-retrovirales puede prolongar la supervivencia y mejorar la calidad de vida en los pacientes con SK asociado a SIDA. De hecho, la introducción de la triple terapia virostática en el manejo de la infección por VIH, ha disminuido dramáticamente la incidencia de SK asociado a SIDA. Asimismo,

como hemos comentado, la combinación de zidovudina con el IFN-α ha mostrado ser útil en el manejo paliativo del SK asociado a SIDA. De estos resultados se deduce que la terapia antiretroviral por sí misma podría ser suficiente para prevenir la aparición del SK en estos pacientes.

Si el VHH-8 es el agente etiológico del SK, es lógico pensar que el tratamiento con medicaciones antiherpéticas podría ser útil. Sin embargo, la infección de las células tumorales del SK es mayoritariamente de tipo latente y no lítica, por lo que la terapia con antiherpéticos no suele ser efectiva (Andrew 2002).

Gracias al establecimiento de modelos animales a partir de los cuales se han conseguido cultivos de células fusiformes del SK, se han podido realizar varios estudios terapéuticos experimentales. Evidentemente la mayoría de ellos se han llevado a cabo con moléculas que interactúan con las proteínas que toman parte en la regulación del crecimiento, quimiotaxis y angiogénesis de las células del SK.

Recientemente se ha demostrado que la cadena β de la gonadotropina coriónica humana (β-HCG) puede destruir células malignas de una línea inmortalizada del SK-SIDA y también a las células derivadas de lesiones de SK que han crecido en cultivos a corto plazo (Lunardi-Iskandar 1995). Aparentemente la hormona induce la muerte celular *in vitro* e *in vivo* a través de mecanismos que estimulan la apoptosis. Sus efectos se encuentran restringidos a las células proliferantes del SK y no afectan a las células endoteliales normales. La inyección intralesional en pacientes con SK asociado a SIDA induce una regresión dosis-dependiente de las lesiones. De todas formas, son necesarios más

experimentos clínicos para poder determinar con exactitud la eficacia y, por lo tanto, la futura aplicabilidad de estas modalidades terapéuticas experimentales.

En general, las guías de tratamiento del SK yatrogénico no son distintas que las de las otras variantes del SK, y evidentemente la primera línea de tratamiento es retirar o disminuir la terapia inmunosupresora. Esto conlleva una desaparición completa de las lesiones del SK en más del 30% de los pacientes. No obstante, la decisión de disminuir o retirar los fármacos inmunosupresores puede ser compleja, ya que deben tomarse en consideración en primer lugar la severidad y extensión del SK, así como la importancia de mantener la medicación inmunosupresora para poder mantener un órgano trasplantado que puede salvar la vida al paciente.

Para intentar evaluar la evolución de los pacientes y eficacia de las diferentes combinaciones terapéuticas se utiliza un sistema de estadiaje del SK, distinto para la forma clásica (Weiss 2001) y para la asociada a SIDA (Krown 1989).

* SISTEMA DE ESTADIAJE PARA EL SK CLÁSICO

Estadio I: Cutáneo, localmente indolente.

Estadio II: Cutáneo, localmente agresivo, con o sin ganglios linfáticos regionales.

Estadio III: Afectación mucocutánea generalizada, o de ganglios linfáticos, o ambas.

Estadio IV: Afectación visceral.

A: Sin síntomas o signos sistémicos.

B: Signos sistémicos (10% de pérdida de peso o fiebre oral de más de 38ºC no relacionada con una fuente de infección identificable de más de 2 semanas).

*** SISTEMA DE ESTADIAJE PARA EL SK ASOCIADO A SIDA**

PARÁMETRO	BAJO RIESGO	ALTO RIESGO
Tumor (T)	Confinado a la piel y/o ganglios linfáticos y/o enfermedad oral mínima.	Edema o ulceración asociadas al tumor; SK oral extenso; SK en visceras no nodales.
Sistema inmune (I)	Células CD4 > 200/µl	Células CD4 < 200/µl
Enf. sistémica (S)	No evidencia de infecciones oportunistas o aftas; No síntomas "B"; Estatus de funcionamiento > 70 (Karnovsky)	Historia de infecciones oportunistas y/o aftas; Síntomas "B"; Estatus de funcionamiento < 70 (Karnovsky); Otras enfermedades relacionadas con el HIV (p.e., linfoma).

III. RESULTADOS

1. PRIMER RESULTADO

DIFFERENTIAL EXPRESSION OF C-MET IN KAPOSI'S SARCOMA ACCORDING TO PROGRESSION STAGE AND HIV INFECTION STATUS

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1.1. ABSTRACT

Several cytokines, growth factors and the HIV transactivator tat have been shown to be involved in the pathogenesis of Kaposi's sarcoma (KS). Hepatocyte growth factor/scatter factor (HGF) is an angiogenic cytokine that stimulates proliferation of spindle cells cultured from human KS lesions. The receptor for HGF, the c-Met protein, is expressed by endothelial cells, dermal dendrocytes and KS tumor cells both *in vitro* and *in vivo*. KS cells synthesize and secrete HGF and express the hepatocyte growth factor receptor (c-Met), thus providing an autocrine loop for tumor proliferation and neovascularization which can be enhanced by proinflammatory cytokines. We studied the immunohistochemical expression of c-Met in 40 cases of classical Kaposi's sarcoma (C-KS) and AIDS-associated cutaneous Kaposi's sarcoma (AIDS-KS), including 22 plaque stage lesions (12 AIDS-KS cases) and 18 tumor stage lesions (7 AIDS-KS cases). Statistically significant differences in the average intensity of immunohistochemical staining according to the type of lesions (progression stages) and the serologic status of the patients were identified. The staining intensity of c-Met was stronger in tumors than in plaques. When only plaques were taken into consideration, the mean staining score was nearly twice as high in C-KS as in AIDS-KS.

1.2. INTRODUCTION

Kaposi's sarcoma (KS) is an angioproliferative disease with a complex pathogenesis involving a disordered cytokine network, immune alterations and active infection with HHV-8.[1-6] Four epidemiological forms of Kaposi's sarcoma (KS) have been described: classic KS (C-KS), African-endemic KS, immunosuppressive drug-related KS, and acquired immune deficiency syndrome-related KS (AIDS-KS). C-KS affects mainly elderly men of Mediterranean origin, while AIDS-KS is predominantly seen in HIV-infected homosexual men and is typically associated with an aggressive course.[1] Histologic findings shared by all forms of KS include prominent angiogenesis and proliferation of spindle-shaped cells, considered to be the tumor cells of KS.

One of the most fascinating features of KS is its binomial (hyperplastic/neoplastic) nature. Thus, KS seems to begin as a reactive vascular proliferation due to an unbalanced cytokine network,[1] whereas in advanced stages, it behaves as a multifocal neoplasm, and a monoclonal origin of some multicentric lesions has been demonstrated.[7] The different biological behavior of KS according to its epidemiological presentations and stages might be related to the degree of immune response impairment in the host and/or to the presence of specific alterations in the mechanisms controlling tumor growth and development, such as cytokine/growth factor overstimulation or escape from regulated cell death (apoptosis).

Hepatocyte growth factor/scatter factor (HGF) is a pleiotropic cytokine that stimulates cell motility, proliferation and invasiveness of epithelial and cancer cells. These responses are transduced through the c-Met proto-oncogene product, a transmembrane tyrosine kinase

that functions as the HGF receptor. HGF activates migration and proliferation of endothelial cells[8] and induces a dose-dependent angiogenic response *in vivo*,[9,10] which is enhanced by heparin and is mediated, at least in part, by platelet activating factor (PAF) synthesized from infiltrating macrophages.[10] The potent angiogenic activity of HGF is mediated primarily through direct actions on vascular endothelial cells, that include stimulation of cell migration, proliferation, protease production, invasion, and organization into capillary-like tubes *in vitro*.[9] HGF is overexpressed in invasive human cancers, relative to non-invasive cancers and benign conditions, and may function as a tumor progression factor, in part by stimulating tumor cell invasiveness and in part by stimulating angiogenesis.[11] Moreover, HGF could act as an indirect angiogenic factor through autocrine induction of VEGF expression and secretion, in malignant gliomas and other neoplasms.[12]

High levels of HGF expression have been found in human sporadic KS specimens, as well as in spindle cells cultured from human lesions.[13] In addition, human lesion-derived spindle cells synthesize and secrete biologically active hepatocyte growth factor and express the hepatocyte growth factor receptor (c-Met).[14]

Since there is very limited information on the immunohistochemical expression of c-Met in different stages of AIDS-associated and classic KS lesions, we have studied the immunohistochemical expression of c-Met using a specific monoclonal antibody.

1.3. MATERIAL AND METHODS

Human tissue samples

Skin biopsy paraffin blocks were retrieved from the files of the Department of Pathology at the Hospital Universitari Germans Trias i Pujol, Badalona, Spain. The specimens corresponded to 40 randomly selected cases of C-KS and AIDS-KS, including 22 cases of plaque stage KS (10 C-KS and 12 AIDS-KS) and 18 cases of tumor stage KS (11 C-KS and 7 AIDS-KS). The stage was determined by histopathologic study of sections stained with hematoxylin and eosin. A diagnosis of plaque stage KS was made when most of the lesion consisted of malformed vascular channels which dissected collagen fibers and contained only isolated spindle-shaped cells or small groups of them. A diagnosis of nodular or tumor phase KS was made when the entire lesion or most of it showed a compact proliferation of spindle-shaped cells with an intersecting fascicle-like pattern and some inflammatory cells, erythrocytes, or telangiectatic spaces. All tissue specimens were fixed in neutral-buffered formalin and routinely processed.

Antibodies and immunohistochemical studies

Immunohistochemical detection of c-Met was performed with a mouse monoclonal anti-human c-Met antibody from Novocastra, (Newcastle upon Tyne, UK) diluted 1:20 with phosphate-buffered saline (PBS). Five-μm sections were cut from the paraffin blocks, deparaffinized, hydrated, immersed in buffered citrate and autoclaved. Afterwards, the sections were incubated for 30 min in rabbit serum. Incubations with primary antibodies were carried out for 22h at room temperature. Slides were washed and incubated with biotinylated rabbit antimouse IgG antibodies at a 1:700 dilution and then incubated in PBS/6% hydrogen periode for 15 min at room temperature before avidin-biotin peroxidase complex addition (Dakopatts, Glostrup, Denmark). The chromogen 3,3' – diaminobenzidine tetrachloride (Serva, Heidelberg, Germany) was used, and counterstaining was performed with Harris hematoxylin. A nonimmune mouse serum was used as a negative control ill this protocol.

Immunostaining results were scored taking into account the percentage of positive tumor cells and the reaction intensity. The percentage of positive tumor cells was graded from 0 to 4 as follows: 0, none to 5%; 1, 5% to 35%; 2, 35% to 65%; 3, 65% to 95%; and 4, 95% to 100%. The intensity of immunostaining was rated as follows: 0, none; 1, weak; 2, moderate; and 3, intense. Specimens were considered to be immunopositive when at least 5% of cells showed clear evidence of immunostaining. Since KS lesions tend to be heterogeneous, a score was calculated in which the percent positive rating was multiplied by the intensity rating for statistical analysis purposes. In this scoring system, each component of the tumor was scored independently and the results were added up. For

instance, a biopsy specimen in which 25% of cells stained intensely (1x3=3) and 50% had a moderate staining intensity (2x2=4) was assigned a global score of 3+4=7. The reproducibility of the results was confirmed by comparison of the scores assigned by two different researchers (M.T.F.F and L.P.). The score was calculated on 6 to 10 representative fields after examination of the totality of tumor present in one section for each case. The smallest size of the biopsy specimens included in the study was that of a punch-biopsy measuring 4 mm in diameter and 5 mm in depth. All tissue sections contained non-tumor cells such as epidermal and adnexal cells, melanocytes, as well as arrectores pili muscles or nerves in some cases, which served as internal positive and negative controls for the assessment of antibody specificity and epitope inmunopreservation.

Statistical analysis

Statistical significance for differences observed in the staining scores between C-KS and AIDS-KS lesions and between plaque lesions and tumor lesions was determined using analysis of variance. When variances were not homogeneous or samples were not normally distributed, the Kruskal-Wallis test was used. Differences between groups were considered to be statistically significant when time p-value was less than 0,05.

1.4. RESULTS

The expression of c-Met by non-tumor cells was moderate to intense in the basal and suprabasal layers of the epidermis. The granular layer showed a weaker staining, except in areas of epidermal hyperplasia, where an intense positivity was present throughout the epidermis. The average intensity of the epidermal staining in the basal layer was similar in all the cases studied, was not correlated with the immunostaining scores assigned to KS areas, and was used as an internal control of the quality of immunostaining. The expression of c-Met was strong in the secretory coil portion of sweat glands, and weak to absent in the intraepidermal ducts. The endothelial cells in non-neoplastic vessels located in the papillary dermis or distant from the tumor were negative for c-Met expression, which was moderate to intense in reactive endothelial cells corresponding to vessels in areas of inflammation, or adjacent to the tumor. A skin biopsy specimen corresponding to a case of angiolymphoid hyperplasia with eosinophilia which was included in the study as a control was assigned an immunostaining score of 12, thus confirming our impression that c-Met was overexpressed in reactive or proliferative endothelium. There was moderate c-Met expression in the smooth muscle cells of arteriolar walls.

The expression of c-Met was weak in KS plaque lesions and moderate or intense in most tumor lesions. There were some variations in the intensity between adjacent cells in the same area and from one area to another, which were scored taking into consideration the approximate percentages of cells corresponding to each intensity, as detailed in the Material and methods section. The results of immunostaining scores are detailed in Table 1.

The mean scores were significantly lower in plaque lesions than in tumor lesions when all cases were grouped together irrespective of the HIV status of the patients (4.6±2.3 (mean±s.d.) vs. 7.5±.2.6, $p<0.0019$). The differences remained statistically significant when cases were stratified according to their serologic status in AlDS-KS (3.4±1.9 vs. 7.9±2.1, $p<0.0011$) but not in C-KS (6.0±2.0 vs. 7.2±3.1, $p= 0.4406$).

The mean staining scores of plaque lesions were significantly lower in AIDS-KS (Fig. 1) than in C-KS cases (Fig. 2) (3.4±1.9 vs. 6.0±2.0, $p<0.0074$), but there were no significant differences between AIDS-KS and C-KS in the mean scores of tumors (Fig. 3) (7.9±2.1 vs. 7.2±3.1, $p=0.6595$, or in those of all lesions grouped together irrespective of their histopathological stage (5.2±3.0 vs. 6.6±2.5, $p = 0.1301$).

In summary, the intensity of c-Met staining was weaker in AIDS-KS initial stages than in C-KS initial stages, and increased in correlation with the degree of tumor cell proliferation in KS lesions (plaques<tumors). The differences in mean scores between plaques and tumors were not statistically significant in C-KS cases, which tended to show higher scores overall. This can be expressed as follows: AIDS-KS plaques << C-KS plaques < C-KS tumors ≈ AIDS-KS tumors (Fig. 4).

Table 1. Results of immunostaining scores

		Case No.	c-Met
Plaques	AIDS-KS	1	7
		2	3
		3	3
		4	3
		5	1
		6	3
		7	1
		8	4
		9	6
		10	4
		11	1
		12	5
	C-KS	13	8
		14	6
		15	6
		16	5
		17	3
		18	6
		19	8
		20	10
		21	5
		22	4
Tumors	AIDS-KS	23	4
		24	7
		25	8
		26	10
		27	10
		28	6
		29	9
	C-KS	30	6
		31	9
		32	4
		33	4
		34	4
		35	10
		36	9
		37	6
		38	12
		39	10
		40	5

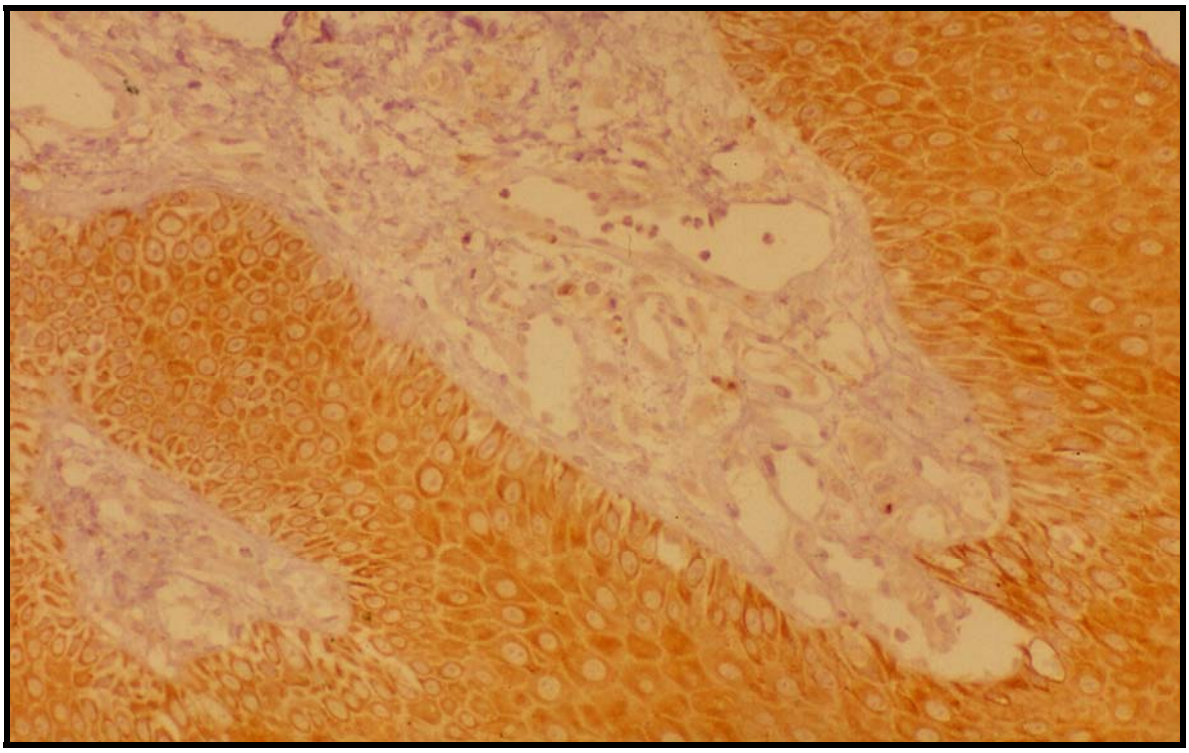

Fig. 1. C-Met immunohistochemical stain of an AIDS-KS plaque lesion, showing weak staining.

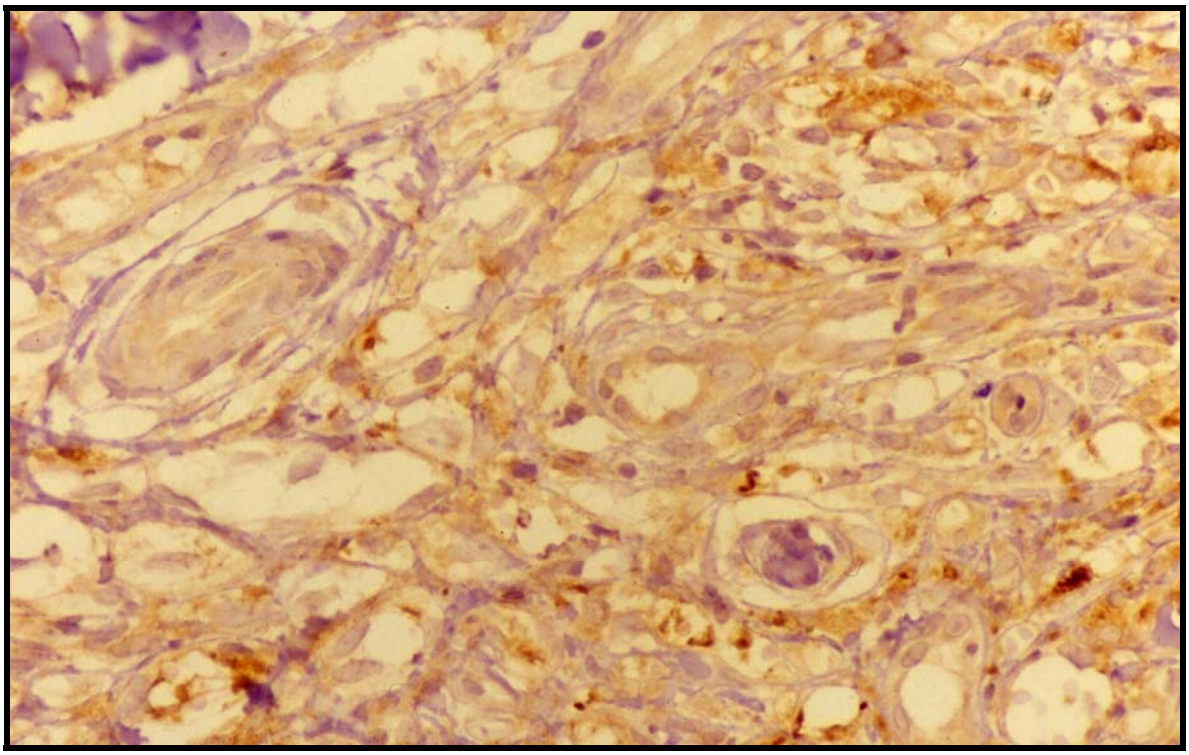

Fig. 2. C-Met immunohistochemical stain of a C-KS plaque lesion. The mean intensity of staining in C-SK plaques was higher than in AIDS-KS.

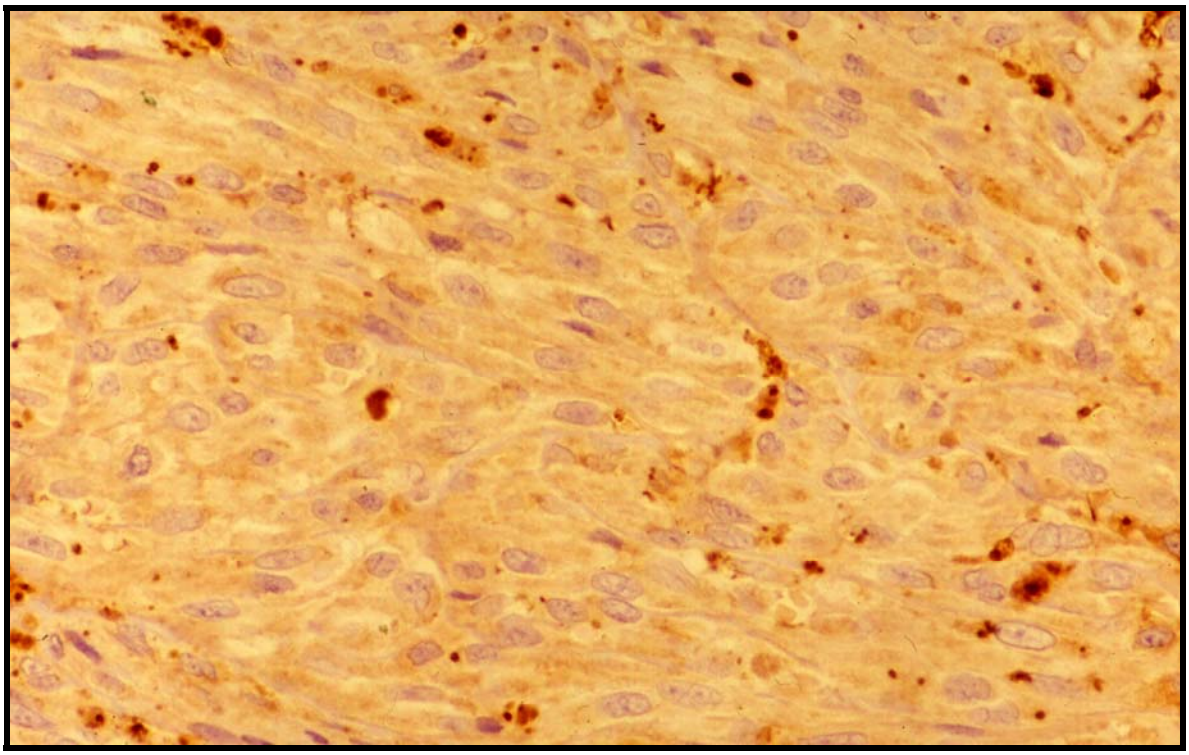

Fig. 3. C-Met immunohistochemical stain of an AIDS-KS tumor lesion. The staining score in tumors was higher than in plaques, irrespective of HIV status.

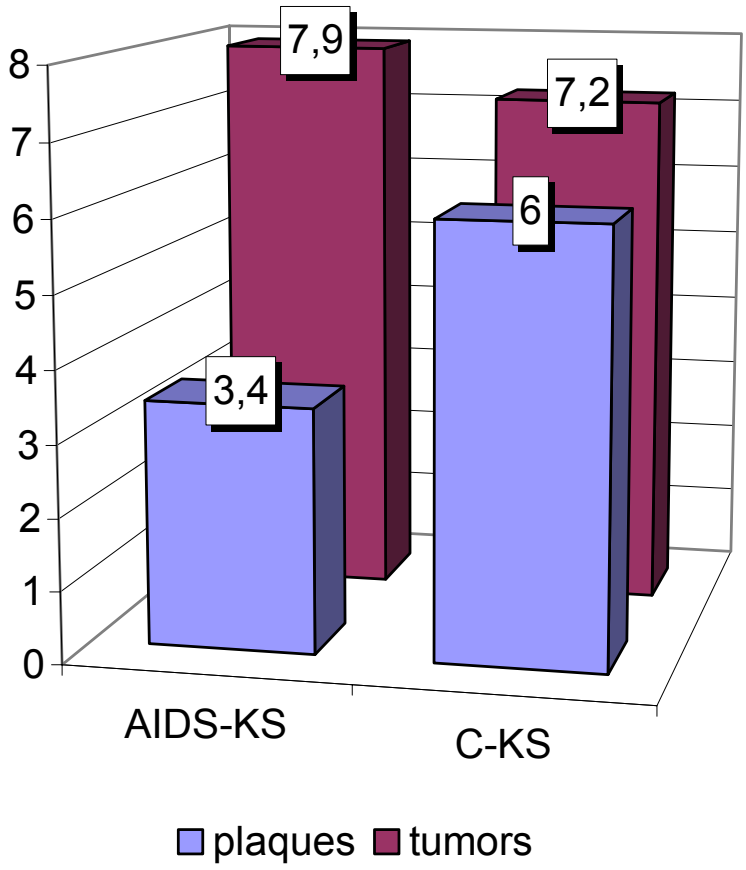

Fig 4. Mean c-Met staining scores.

1.5. DISCUSSION

Several cytokines, growth factors and the HIV transactivator tat have been shown to be involved in the pathogenesis of Kaposi's sarcoma. On the other hand, all the epidemiological forms of KS seem to be linked to HHV8 infection. It is surmised that HHV8 may exert its effects either directly through the encoding of growth-promoting and antiapoptotic proteins or indirectly through the induction of cytokines and angiogenic proteins synthesis in infected and inflammatory cells.[6] Amongst the latter, the most relevant are γ-interferon (γIFN), basic fibroblastic growth factor (bFGF or FGF-2) and vascular endothelial growth factor (VEGF), which are responsible for the hyperplastic angiogenic proliferation so characteristic of KS initial stages and the spindle cell phenotype.[6]

BK virus (BKV)/tat transgenic mice develop KS-like lesions, and spindle-shaped cells of a murine KS-like line (TTB) derived from these lesions co-express HGF and its receptor, c-Met.[14] TTB cells co-express antigens specific for endothelial, smooth muscle and antigen-presenting cells, suggesting that they represent poorly differentiated vascular precursors, and synthesize a complex mixture of angiogenic factors, including FGF-2, VEGF and placental growth factor, in addition to HGF.[15] An autocrine HGF/c-Met loop sustains spindle cell proliferation of TTB cells *in vitro;* in agreement with this, an antisense oligomer targeted against HGF markedly inhibits cell growth.[14] Induction of the HGF/c-Met autocrine loop upon exposure of TTB cells to the proinflammatory cytokine interleukin 1 (IL-1) would explain the appearance of multiple foci of uncontrolled growth.[14] In addition, due to its angiogenic effect, HGF may also sustain the

neovascularization so typical of Kaposi's sarcoma lesions. When administered together *in vitro,* HGF and VEGF have synergistic effects, which can be reproduced *in vivo* by HGF-induced up-regulation of VEGF in vascular smooth muscle cells.[16] FGF-2 stimulates TTB cell migration and promotes polarization of urokinase-type plasminogen activator receptor (uPAR) at the leading edge of migrating cells. [17] FGF2 stimulated migration is blocked both by antibodies against urokinase-type plasminogen activator or uPAR and by neutralizing anti-HGF antibodies. The latter also inhibits uPAR relocalization at the cell surface of FGF-2-treated TTB cells. This points to a cross-talk between FGF-2 and HGF that might mediate TTB cell migration by modulating the localization of cell surface uPAR.[17]

Our findings suggest that there is an enhanced upregulation of c-Met expression in plaque stage C-KS with respect to AIDS-KS. This upregulation of c-Met increases to a maximum in tumor stages, irrespective of HIV serologic status. The overexpression of c-Met might be involved in several paracrine and autocrine loops leading to increased angiogenesis, spindle cell proliferation and migration, resistance to apoptosis, and eventually KS progression. The intensity of c-Met expression in KS biopsy specimens in our study might be correlated with the time of evolution of cutaneous KS lesions; even though their size was similar, the evolution of C-KS lesions is usually slower, and macules of AIDS-KS tend to be biopsied earlier for diagnostic purposes. On the other hand, minimal overexpression of c-Met in plaque stage AIDS-KS might imply that in the initial stages of AIDS-KS development other cytokine loops related to HIV infection (e.g., γIFN or HIV tat) are more relevant than IL-1 for angiogenesis and spindle cell proliferation. Recombinant HIV-tat has a basic domain similar to those of several heparin binding

angiogenic factors, including FGF, VEGF and HGF, which would account for its angiogenic activity, both *in vivo* and *in vitro,* being modulated by heparin and heparan-sulfate.[18]. In tumor stages, IL-I would overdrive c-Met expression and HGF-mediated proliferation, irrespective of HIV infection. Other angiogenic factors involved in KS pathogenesis, such as FGF-2 and VEGF, might act synergistically with HGF/c-Met to promote KS progression.

HGF may also block the induction of apoptosis by various DNA damaging-agents, including cytotoxic agents in MDA-MB-453 human breast cancer cells, and possibly in KS cell lines.[19] This mechanism would provide an additional protection of KS cells against apoptosis and in so doing contribute to KS tumor progression, especially in the more advanced stages.

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2. SEGUNDO RESULTADO

DECREASED IMMUNOREACTIVITY FOR CELL-CYCLE REGULATOR $P27^{KIP1}$ IN KAPOSI'S SARCOMA CORRELATES WITH HIGHER STAGE AND EXTRACUTANEOUS INVOLVEMENT

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2.1. ABSTRACT

A consistent relationship has been established between the development of Kaposi's sarcoma (KS) and human herpes virus-8 (HHV8) infection. HHV8 encoded v-cyclin, through its complexing with cyclin-dependent kinase 6, contributes to the phosphorylation and proteasome-mediated degradation of $p27^{Kip1}$. On the other hand, down-regulation of $p27^{Kip1}$ expression seems to facilitate metastatic dissemination in a variety of human neoplasms. Although the neoplastic nature of KS remains controversial, it has been repeatedly demonstrated that in some patients KS may behave as a malignant neoplasm and follow an ominous course, especially in HIV-positive patients and when associated with extracutaneous involvement. To determine whether decreased $p27^{Kip1}$ levels are also related to more aggressive behaviour in KS, it was decided to investigate $p27^{Kip1}$ immunoreactivity in KS biopsy specimens and its possible changes in relation to cutaneous versus extracutaneous involvement and HIV serological status. Forty-nine cases of KS (29 AIDS-related and 21 classical) corresponding to 30 cutaneous biopsy specimens (ten macules, seven plaques, and 13 tumours) and 19 extracutaneous biopsy specimens were immunostained to determine the expression of $p27^{Kip1}$ and the proIiferalion marker Ki67 antigen. The mean percentages of $p27^{Kip1}$ -positive cells were significantly higher in biopsy specimens from skin lesions (77.8±21.1) than in those from extracutaneous locations (42.0±26.0). Amongst cutaneous lesions, $p27^{Kip1}$ expression was significantly higher in macules (83.8±18.5) and plaques (91.4±6.4) than in turmours (65.8±22.6). Ki67 immunoreactivity showed no correlation with any of the variables studied. These results lend support to the hypothesis that decreased levels of $p27^{Kip1}$, which may have been

brought about by HHV8 infection, play a role in KS progression through its various histopathological stages, to its eventual extracutaneous spread.

Keywords: Kaposi's sarcoma; $p27^{Kip1}$; cell cycle; immunohistochemistry; AIDS; HIV

2.2. INTRODUCTION

Kaposi's sarcoma (KS), an angiohyperplastic disease mediated by inflammatory cytokines and angiogenic factors, is triggered or amplified by infection with the KS-associated human herpesvirus-8 (HHV8) [1]. Four clinical-epidemiological forms of KS have been described so far: a classical form (C-KS), African-endemic KS, iatrogenic or immunosuppressive drug-related KS, and acquired immune deficiency syndrome (AIDS)-related KS (AIDS-KS) [2]. Even though AIDS-KS is more aggressive than the other types, the fact that immunological and histopathological features are shared by all KS forms suggests common aetiological and pathogenic factors. The human immuno-deficiency virus type-1 (HIV-1) tat protein appears to be responsible for the higher grade of aggressiveness of AIDS-KS compared with the other forms [1]. In advanced stages, KS may behave as a multifocal neoplasm, a monoclonal origin of multicentric lesions having been demonstrated in some cases [3,4]. On the other hand, a polyclonal origin of multiple KS lesions derived from an individual patient has also been shown [3]. KS involvement is usually limited to the skin, but in aggressive cases it may disseminate to mucous membranes and internal organs. Whether extracutaneous involvement represents metastatic spread or multifocality is not clear. Alterations of the oncoprotein networks that control cell proliferation and apoptosis would provide neoplastic stimuli for the development, progression, and aggressive behaviour of KS. Previous studies have shown that alterations in the expression of molecules involved in cell-cycle and apoptosis control, such as p53 [5,6], cyclin D1 [7], and Bcl-2 [8], may be related to KS tumour progression.

Progression through the cell cycle is governed by a series of cyclin-dependent kinases (CDKs), which are activated by binding to cyclin proteins, regulated by phosphorylation, and inhibited by CDK inhibitors [9]. Two families of CDK inhibitors that modulate the activity of cyclin-CDK complexes have been described: the Cip/Kip and Ink 4 families [9]. The Cip/Kip family includes $p21^{Cip1}$, $p27^{Kip1}$, and $p57^{Kip2}$ proteins [9,10]. CDK inhibitor $p27^{Kip1}$ seems to provide a central signal which coordinated varied inputs from the extracellular environment and serves as a threshold for progression to the S phase or exiting the cell cycle [2]. $p27^{Kip1}$ regulates cell proliferation by binding and inhibiting G1 cyclin-CDK complexes and therefore negatively regulating progression through the G1 and S phases of the cell cycle [9]. Additionally, $p27^{Kip1}$ associates with cyclin E-CDK2, cyclin A-CDK2, and cyclin D-CDK4 complexes, abrogating their activity [11,12]. Moreover, high levels of $p27^{Kip1}$ are found in quiescent cells, suggesting that it can play a role in maintaining cells in the G0 phase [10]. $p27^{Kip1}$ expression is regulated by cell contact inhibition and by specific growth factors, such as transforming growth factor β (TGF-β) [12,13]. Cell-cell contact (such as that occurring at the confluence of epithelial or mesenchymal cells in culture) and loss of cell adhesion (as in cells grown in suspension) have been shown to up-regulate $p27^{Kip1}$ levels [14]. On the other hand, it has been suggested that loss of $p27^{Kip1}$ protein expression may result in tumour development and-/or progression [9,13,15-23].

A large number of studies have characterized $p27^{Kip1}$ as an independent prognostic factor in various human cancers, including breast [15], colon [17-19], stomach [20], prostate [16], non-small cell lung [13], endocrine [21], ovarian [22], and oral cavity [23] carcinomas. A comparative study of $p27^{Kip1}$ expression in primary colorectal carcinomas and their

metastases showed an important reduction in $p27^{Kip1}$ expression in the metastatic foci compared with their primary tumours [17]. This suggests that the development of metastases may be facilitated by $p27^{Kip1}$ down-regulation in circulating tumour cells, which would thus, become able to grow in an altered extracellular matrix, or acquire abnormal intercellular adhesion properties.

The anti-$p27^{Kip1}$ immunohistochemical labelling index is closely correlated with the level of $p27^{Kip1}$ expression as measured by western blotting [13]. To our knowledge, immunohistochemical expression of $p27^{Kip1}$ in KS biopsy specimens and its possible alterations interelation to cutaneous and extracutaneous involvement and HIV serological status have not previously been systematically studied. The aim of the present investigation was to identify potential alterations of $p27^{Kip1}$ expression in KS cells in relation to HIV serological status, KS histopathological stage, and KS cell immunostaining for Ki67. In addition, we sought to determine whether the decrease in $p27^{Kip1}$ protein expression levels is related to extracutaneous involvement in KS, as is the case in several other types of neoplasms when they metastasize [13,15-22].

2.3. MATERIALS AND METHODS

Human tissue samples

Skin biopsy paraffin blocks were retrieved from the files of the Department of Pathology of Hospital Universitari Germans Trias i Pujol, Badalona, Spain, and the Department of Pathology of Hospital de la Santa Creu i Sant Pau, Barcelona, Spain. We evaluated 49 cases of KS, of which 28 corresponded to AIDS-KS and 21 to C-KS. Four of the 49 cases followed an aggressive course and two showed early extracutaneous involvement. The 49 cases provided 30 skin biopsy specimens (ten macules, seven plaques, and 13 tumours) and 19 biopsy specimens from extracutaneous locations [eight from the gastrointestinal tract (including two cases of aggressive C-KS), five from oral mucosa or conjunctiva, three from lymph nodes, one from the larynx, one from the lung, and one from soft tissues].

The stage was determined by histopathological study of haematoxylin and eosin-stained sections [24]. Macular stage lesions consisted of a superficial or mid-dermal proliferation of collagen-dissecting jagged capillary vessels that disposed themselves around normal dermal structures. In some instances, the newly formed vessels were confluent, but a spindle cell component was always inconspicuous. A diagnosis of KS plaque stage was made when the lesion consisted of a proliferation of malformed vascular channels that dissected the collagen fibres and contained only isolated spindle-shaped cells or small groups of them. A diagnosis of nodular or tumour phase was made when the entire lesion, or most of it, showed a compact proliferation of spindle-shaped cells with an intersecting fascicle-like pattern, interrupted only by some inflammatory cells, erythrocytes, or

telangiectatic spaces. All tissue specimens had been fixed in neutral-buffered formalin and routinely processed.

Antibodies and immunohistochemical studies

Immunohistochemical studies were performed using p27 protein mouse monoclonal antibody, clone 1B4 [Novocastra, Newcastle, UK; diluted 1:40 with phosphate-buffered saline (PBS)] and NCL-Ki67-MM1 mouse monoclonal antibody (Novocastra; diluted 1:50 with PBS). Five-micrometre sections were depar-affinized, hydrated, immesed in buffered citrate, and autoclaved. Afterwards, the sections were incubated for 30 min in rabbit serum. Incubations with primary antibodies were carried out for 22 h at room temperature. Slides were washed and incubated with biotinylated rabbit anti-mouse Ig antibodies at a 1:700 dilution and then incubated in PBS-6% hydrogen peroxide for 15 min at room temperature before avidin-biotin peroxidase complex addition (Dakopatts, Glostrup, Denmark). The chromogen 3,3'-diamino-benzidine tetrahydrochloride (Serva, Heidelberg, Germany) was applied, and counterstaining was performed with Harris' haematoxylin. A non-immune mouse serum was used as a negative control in this protocol.

We considered elongated cells lining abnormal KS vessels or spindle cells within the lesions as tumour cells in KS lesions. The percentages of $p27^{Kip1}$ - and Ki67-positive cells were assessed independently by two researchers in at least 500 tumour cells and/or 20 fields in macule and plaque stage lesions, where tumour cells were less abundant. At least 200 cells were counted in every case. The quotients (positive tumour cells/total number of tumour cells counted) were converted to percentages and rounded to the nearest integer. The arithmetic mean of both observers' scores was used for statistical evaluation. The observers' concordance rate was above was above 95%. Two cases in which inter-observer variation exceeded 10% were jointly re-evaluated. In addition, cases were classified as

high or low $p27^{Kip1}$ expressors using 70% as a cut-off, so that high expression was considered to be present in cases showing over 70% $p27^{Kip1}$ -positive nuclei.

Statistical study

The statistical, significance of differences observed between C-KS and AIDS-KS lesions and between plaque lesions and tumour lesions with regard to Ki67 immunostaining scores, KS clinical-epidemiological type, sex of patient, and lesion location and type was determined using analysis of variance. When variances were not homogeneous or samples were not normally distributed, the Mann-Whitney test was used. In addition, $p27^{Kip1}$ expression was compared in the same groups using the cut-off described above and the chi square test for differences between proportions. Differences between groups were considered to be statistically significant when the *p* value was less than 0.05. The pearson correlation coefficient with 95% confidence limits was calculated to evaluate a possible correlation between $p27^{Kip1}$ and Ki67 expression.

2.4. RESULTS

The patient's sex, lesion location, histopathological type, epidemiological classification, and percentages of $p27^{Kip1}$ - and Ki67-positive tumour cells for every case are detailed in Table 1. Even though all instances of extracutaneous KS except one occurred in male patients, no statistically significant differences in $p27^{Kip1}$ expression could be found when cases were classified according to the patient's sex, nor could any sex-related significant differences be found following stratification according to clinical-epidemiological classification of KS or type of skin lesion.

The mean percentages of $p27^{Kip1}$ expression were significantly higher in biopsy specimens from patients with non-aggressive. C-KS (76.9±23.2) (mean ± standard deviation) or aggressive C-KS (71.5±28.5) than in AIDS-KS cases (55.0±9.6; $p<0.04$). The corresponding rates of cases with high $p27^{Kip1}$ expression were 12/17 (71%), 3/4 (75%), and 10/28 (36%) ($p<0.05$). The differences between non-aggressive C-KS and aggressive C-KS were not statistically significant. The mean percentages of $p27^{Kip1}$ expression were significantly higher in biopsy specimens from HIV-negative patients than in the AIDS-KS group (75.9±23.6 vs. 55.0±29.6; $p=0.01$). The corresponding rates of cases with high $p27^{Kip1}$ expression were 15/21 (71%) and 10/28 (36%) ($p=0.01$). The differences were not statistically significant when cases were stratified according to the cutaneous or extracutaneous location of lesions: the mean percentage of $p27^{Kip1}$ expression in cutaneous KS specimens was 76.7 ± 19.4 in cases corresponding to HIV-positive patients and 78.4±22.5 in those from HIV-negative patients (*p* not significant). Thus, major differences in $p27^{Kip1}$ expression seemed to depend on the location of lesions, and overall differences

according to HIV serological status were probably due to over-representation of AIDS-KS (17/19, 89%) in the extracutaneous group.

The mean percentages of $p27^{Kip1}$ expression were significantly higher in biopsy specimens from skin lesions than in those from extracutaneous locations (77.8±21.1 vs. 42.0±26.0, p=0.000003) (Figures 1A-1E). The proportion of cases with high $p27^{Kip1}$ expression (over 70% $p27^{Kip1}$ -positive nuclei of tumour cells) was 21/30 (70%) for skin KS vs. 4/19 (21%) for extracutaneous KS. This difference was statistically significant (p< 0.003).

As regards cutaneous lesions, the mean percentages of $p27^{Kip1}$ expression were significantly higher in skin biopsy specimens corresponding to plaques (91.4±6.4) or macules (83.8±18.5) than in tumours (65.8±22.6; p=0.01) (Figures 1A-1C). The difference between plaques and macules was not statistically significant. The corresponding rates of cases showing high $p27^{Kip1}$ expression were 7/7 (100%), 7/10 (70%), and 7/13 (54%) (p not significant). The mean percentage of $p27^{Kip1}$ expression in non-tumour skin lesions (macules and plaques) was found to be significantly higher than in tumours (86.9±14.9 vs. 65.8±22.6; p<0.004). The corresponding rates of cases showing high $p27^{Kip1}$ expression were 14/17 (82%) and 7/13 (54%) (p not significant). When cases were stratified according to HIV serological status, the differences in the mean percentage of $p27^{Kip1}$ expression between non-tumour skin lesions (macules and plaques) and tumours remained statistically significant only in the HIV-negative group (91.8±7.5 vs. 63.5±24.7; p<0.006). The corresponding rates of cases with high $p27^{Kip1}$ expression were 10/10 (100%) and 4/9 (44%) (p=0.01).

In cases of extracataneous KS, there were no statistically significant differences in $p27^{Kip1}$ expression according.to the origin of the biopsy specimen (oral mucosa and conjunctiva vs. gastrointestinal, lymph, node, and other locations).

The mean percentage of Ki67 expression was 17.1±10.5 (mean±standard deviation). No statistically significant differences were found when cases were stratified according to location, type of lesion, patient's HIV status, sex or any other variable taken into consideration in this study. $p27^{Kip1}$ and Ki67 immunohistochemical scores also failed to show any statistically significant correlation (Figure 2).

In short, a significant down-regulation of $p27^{Kip1}$ immunohistochemical expression was observed in both cutaneous KS tumour lesions and extracutaneous KS lesions. This $p27^{Kip1}$ down-regulation was more prominent biopsy specimens of non-cutaneous KS, irrespective of their anatomical origin. When biopsy specimens were grouped according to location and/or KS histopathological stage, the differences in $p27^{Kip1}$ immunoreactivity according to KS clinical-epidemiological type or HIV serological status were not statistically significant.

Table 1. Kaposi's sarcoma cases: location of lesion, stage, and immunoreactivity for $p27^{Kip1}$ and Ki67

		Case No.	Patient's sex	% of $p27^{Kip1}$ –positive cells	% of Ki67 – positive cells	KS clinical type	Observations
Skin	Macules	1	F	100	7	C	
		2	F	100	23	C	
		3	M	45	37	H	
		4	M	66	28	H	
		5	M	85	10	C	
		6	M	69	41	H	
		7	M	95	17	H	
		8	M	100	4	H	
		9	M	83	2	C	Aggressive course
		10	M	95	39	H	
	Plaques	11	M	90	16	H	
		12	M	90	15	C	
		13	M	100	5	C	Aggressive course
		14	M	100	40	C	
		15	M	87	45	C	
		16	F	83	26	C	
		17	M	90	10	C	
	Tumours	18	M	75	15	H	
		19	F	25	4	C	
		20	M	59	21	C	
		21	F	74	9	C	
		22	F	82	12	H	
		23	F	68	21	C	
		24	M	84	20	H	
		25	M	43	32	H	
		26	M	74	6	C	
		27	M	56	22	C	
		28	M	87	28	C	
		29	F	29	12	C	
		30	F	100	17	C	
Extracutaneous	Oral mucosa and conjuntiva	31	M	81	10	H	
		32	M	61	4	H	
		33	M	47	12	H	
		34	M	12	20	H	
		35	M	35	18	H	
	Gastrointestinal tract, limph, nodes and other locations	36	M	33	19	C	Aggressive course
		37	M	30	8	H	
		38	M	54	15	H	
		39	M	50	16	H	
		40	M	58	10	H	
		41	M	80	8	H	
		42	M	15	10	H	
		43	M	70	19	C	Aggressive course
		44	M	16	6	H	
		45	M	90	15	H	
		46	M	15	21	H	
		47	M	20	18	H	
		48	M	10	14	H	
		49	M	21	11	H	

M= male, F= female, C=classical; H=HIV-associated.

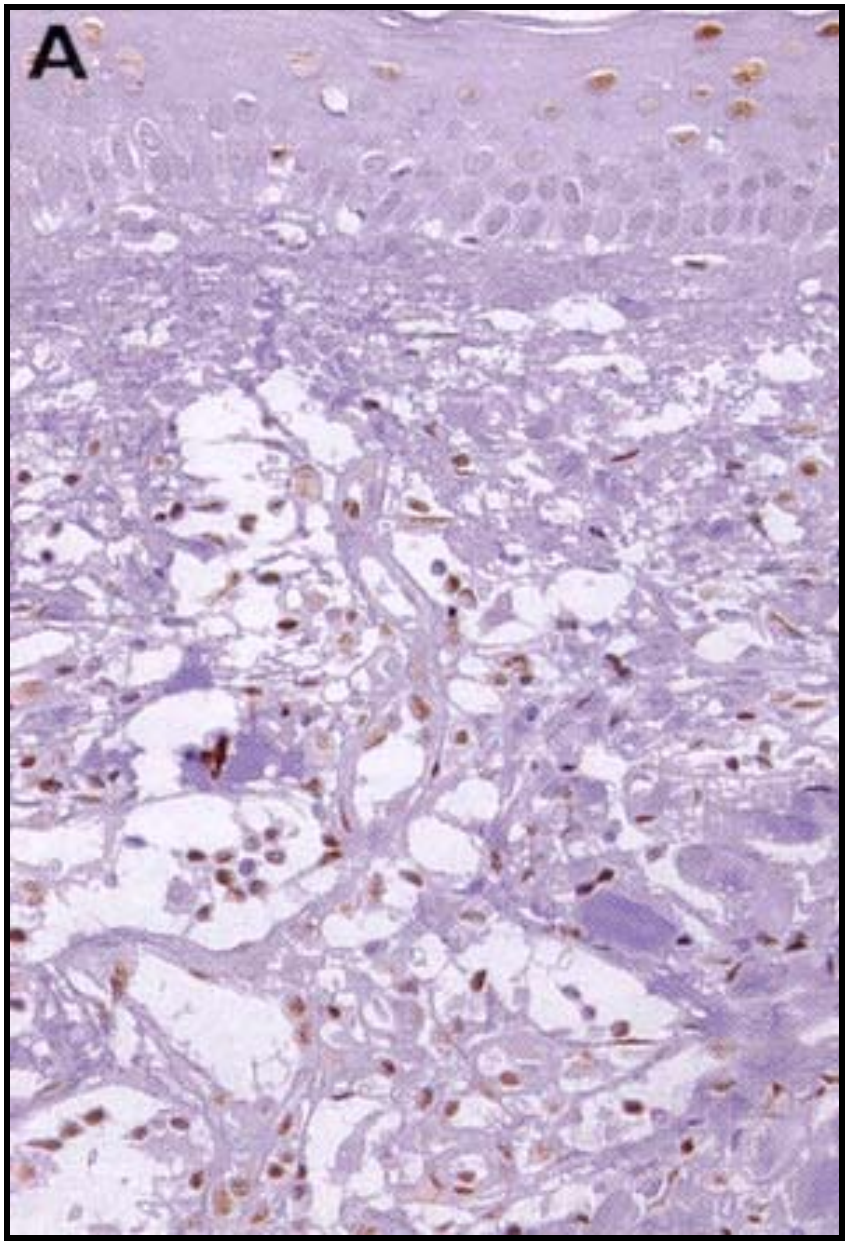

Fig. 1(A). Positive staining for p27 in most nuclei of a macular KS lesion. Note also the staining of some nuclei of suprabasal keratinocytes.

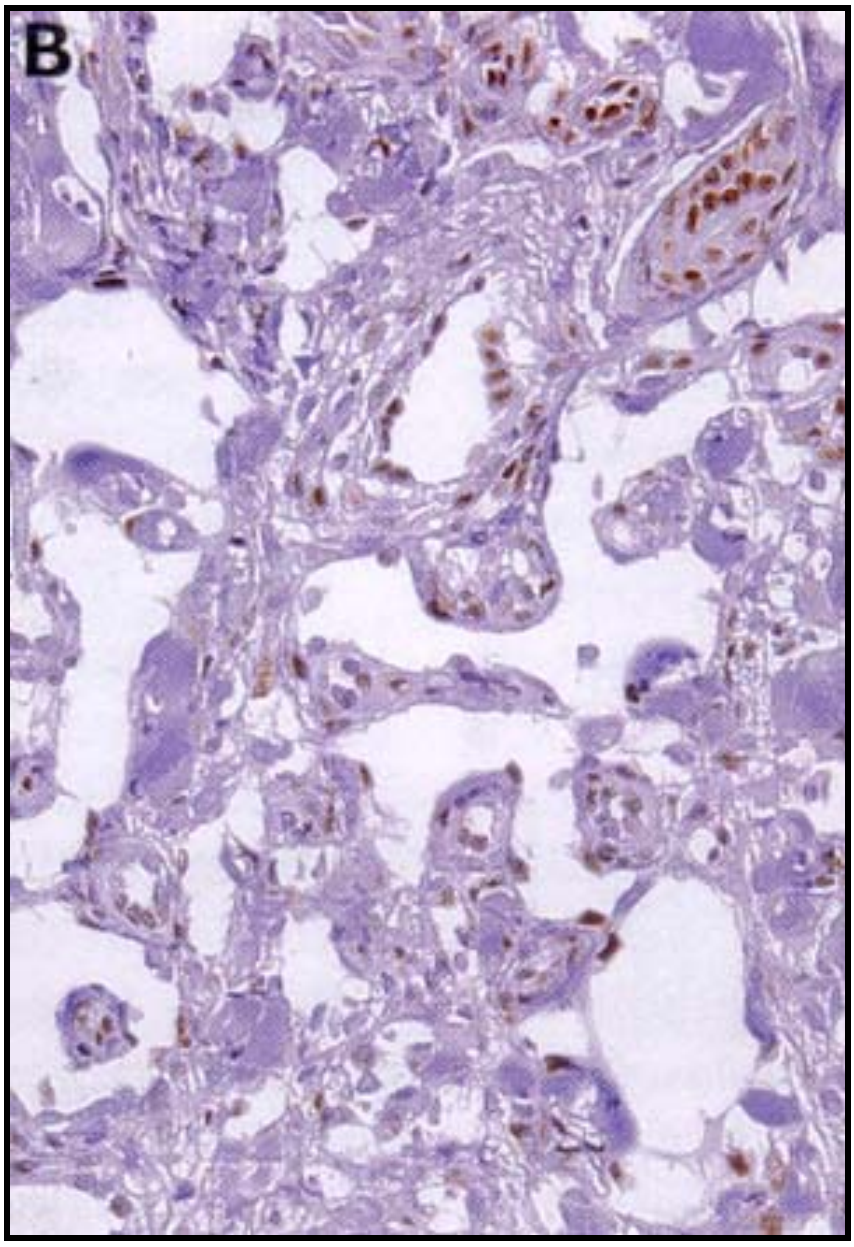

Fig. 1(B). Slight decrease in the total number of positive cells from a KS plaque lesion. A sweat gland duct in the upper left corner also exhibits nuclear staining.

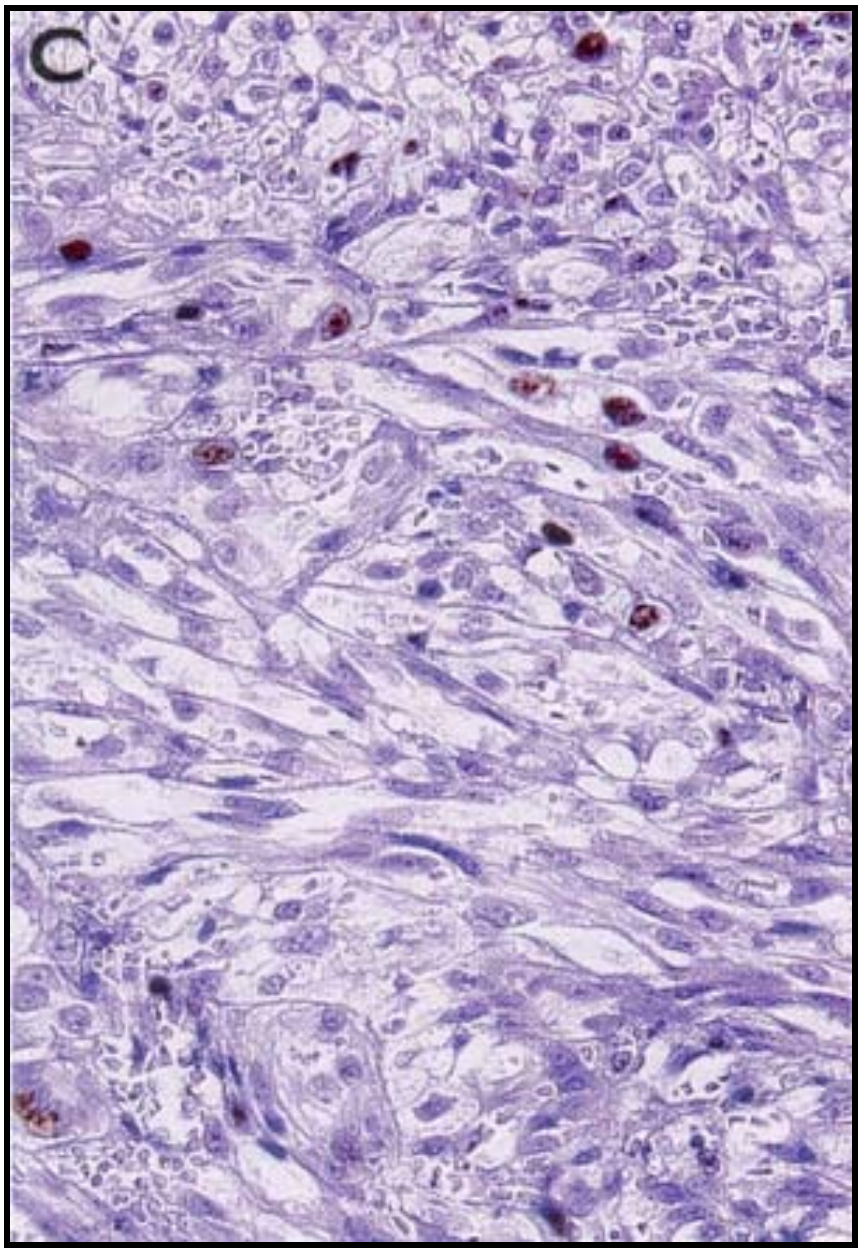

Fig. 1 (C). Tumour KS lesion showing positivity for p27 in a small percentage of nuclei.

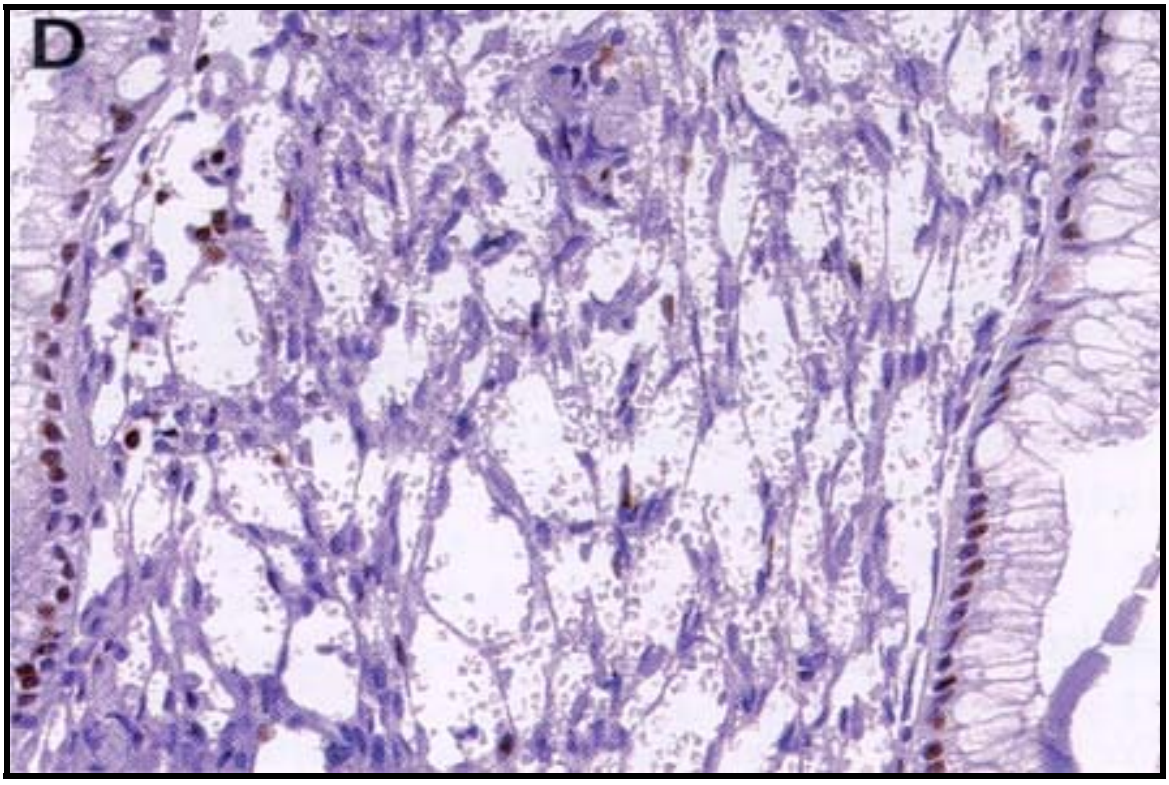

Fig. 1 (D). KS invading the gastric epithelium: p27 expression is present in only a few KS nuclei, whereas most nuclei in gastric glands are positive. (E) KS infiltration of a lymph node: $p27^{Kip1}$ staining is negative in most KS nuclei. Only some lymphocytes from the remains of a lymphoid follicle are positive

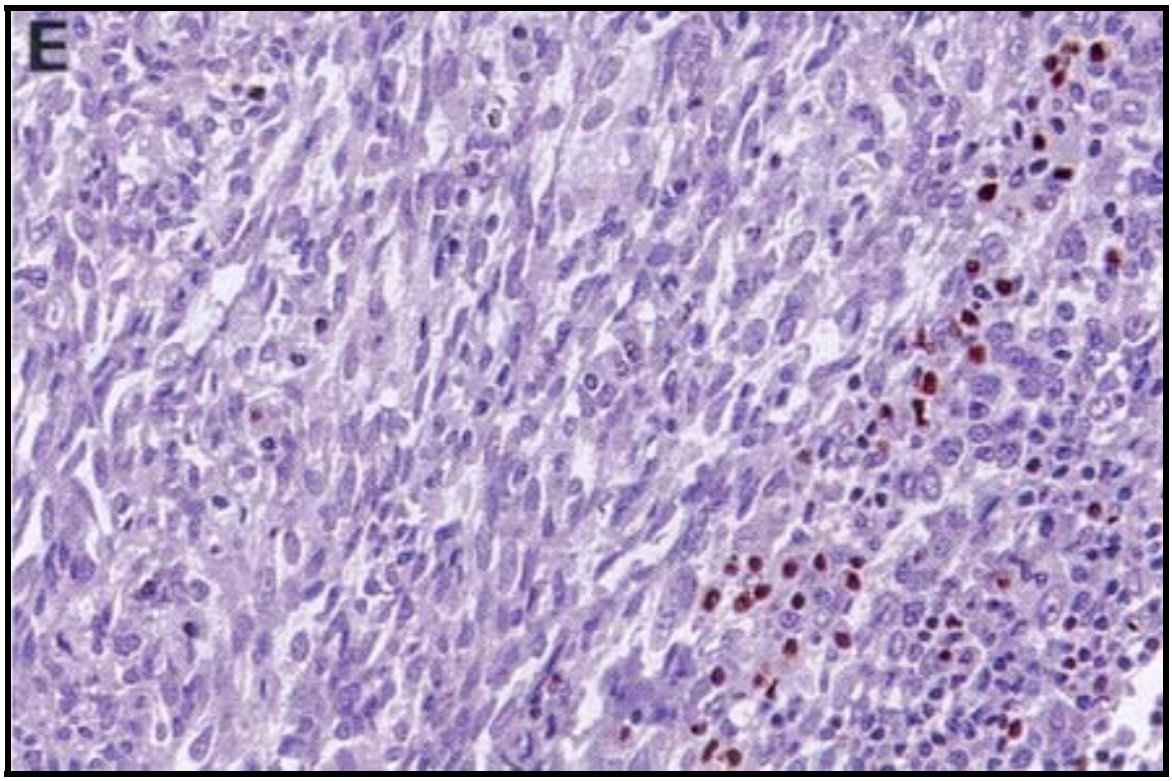

Fig. 1 (E). KS infiltration of a lymph node: $p27^{Kip1}$ staining is negative in most KS nuclei. Only some lymphocytes from the remains of a lymphoid follicle are positive

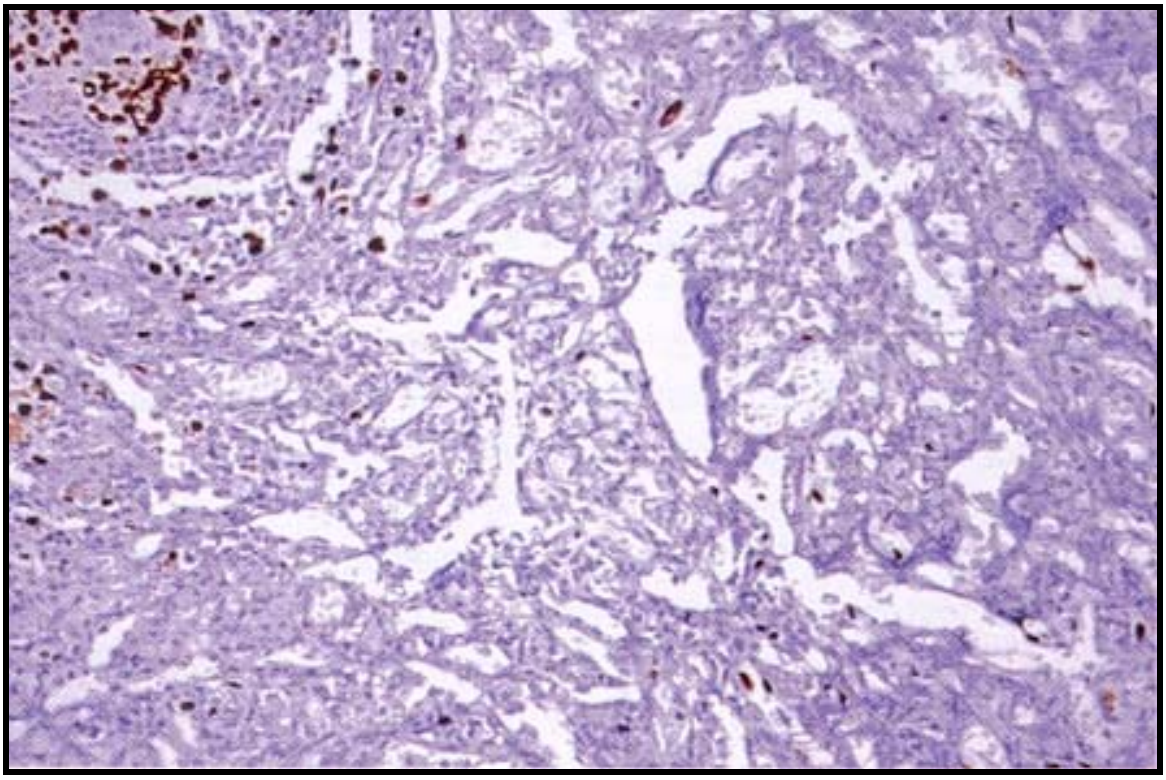

Fig. 2. Ki67 staining is positive in very few KS cells in a lymph node (same biopsy specimen as depicted in Figure 1E). This example illustrates our finding that loss of $p27^{Kip1}$ is not correlated with high Ki67 proliferation index

2.5. DISCUSSION

Our results indicate that there is a relationship between decreased $p27^{Kip1}$ expression and progression of cutaneous KS lesions from macules to tumours. In nodular lesions, the great majority of the cells are KS cells, but in earlier lesions of KS, it is difficult to know, on a cell-by-cell basis, which is a KS abnormal cell and which is a background normal vascular cell or fibroblast. Thus, higher $p27^{Kip1}$ -positive cell counts could be expected in early lesions, so we made every effort to select for counting only KS cells as defined in the Materials and methods section. There were no statistically significant differences in the mean percentages of $p27^{Kip1}$ expression in cutaneous KS lesions between classical and HIV-associated KS cases, Reduction in $p27^{Kip1}$ levels appears, therefore, to be a common but late event in cutaneous KS.

Further reduction in $p27^{Kip1}$ expression in extracutaneous lesions provides additional support for the hypothesis of an inverse correlation between $p27^{Kip1}$ expression and progression of KS. Extracutaneous involvement, an apparently ominous prognostic sign in KS [24], is associated with the lowest $p27^{Kip1}$ expression, regardless of the lesions being located on external mucous membranes such as the oral cavity, or in internal organs such as the lungs. This may indicate that $p27^{Kip1}$ down-regulation facilitates the growth of KS cells in environments other than their primary site, much as happens in some instances of metastatic carcinoma [17].

In various tumours, decrease in $p27^{Kip1}$ levels is due to post-transcriptional protein degradation by the ubiquitin-proteasome pathway [19,25] and, to a lesser extent, by

methylation [26]. Transcriptional and translational control is a further mechanism regulating $p27^{Kip1}$ accumulation during the cycle [16,27], whereas tumour-specific mutations of $p27^{Kip1}$ seem to be exceptional events [12]. In the prostate, both benign hyperplasia and carcinoma are associated with reduced levels of $p27^{Kip1}$ expression, but in carcinoma, messenger RNA (mRNA) levels are similar to those present in the normal prostate. This would indicate that in prostatic carcinoma the suppression of $p27^{Kip1}$ is post-transcriptional, whereas in benign prostatic hyperplasia there is a loss of $p27^{Kip1}$ mRNA secondary to reduced transcription. Moreover, $p27^{Kip1}$ -null mice develop hyperplasia in many tissues without an associated increase in the incidence of neoplasms [28]. These data suggest that reduction of $p27^{Kip1}$ function may lead to either neoplasia or hyperplasia, depending on the causal mechanism.

Recent studies have shed light on the pathways used by viral oncoproteins to inhibit the activities of the CK1s $p21^{Cip1}$ and $p27^{Kip1}$ [29]. As for KS, there is a consistent relationship between HHV8 infection and KS development [1]. HHV8 is a gamma-2 herpes virus that encodes viral effectors homologous to a variety of oncogenes. The latter are able to induce transformation and angiogenesis, evade apoptotic destruction of infected cells [30-33], and favour cell-cycle progression, as does v-cyclin, the viral homologue of cyclin-D [34].

Cyclin behaves differently from typical D-type cyclins. Thus, complexes formed between v-cyclin and CDK6 are resistant to CDK inhibitory proteins, circumventing the G_1 blockade imposed by CK1s. These complexes are able to phosphorylate a wide range of substrates, including $p27^{Kip1}$, and are responsible for the virally induced destabilization of this protein. Phosphorylation of $p27^{Kip1}$ triggers its proteasome-mediated degradation and

leads to a reduction in endogenous $p27^{Kip1}$ levels using the very mechanisms resorted to by many neoplasms. v-Cyclin is not sufficient to enforce DNA synthesis when $p27^{Kip1}$ levels remain elevated. In this way, by down-regulating $p27^{Kip1}$, v-cyclin may lead to the generation of a molecular environment that enables the cell to enter the S phase of its cycle [35,36]. Further studies using *in situ* hybridization to quantify the expression of $p27^{Kip1}$ mRNA would provide additional information on the level of $p27^{Kip1}$ down-regulation and the impact of HHV8-derived v-cyclin on its degradation.

On the other hand, $p27^{Kip1}$ expression in the cutaneous KS lesions studied by us was mostly related to their histopathologicaI stage and largely independent of patients' HIV status, even though differences between non-tumour and tumour lesions lacked statistical significance only in the HIV-positive group. Nevertheless, when the cutaneous and extracutaneous cases were analysed together, the levels of $p27^{Kip1}$ were significantly lower in the HIV-positive group, where most cases of extracutaneous KS belonged. The number of HIV-negative patients with extracutaneous KS in our study was too small to support any hypothesis regarding the possible role of HIV infection in $p27^{Kip1}$ down-regulation.

KS patients show a male predominance [1], which has been accounted for by the protective effect of female sex hormones [37]. Nevertheless, once KS develops, we have found no difference in $p27^{Kip1}$ expression relative to sex.

Finally, some studies have shown a negative correlation between $p27^{Kip1}$ levels and Ki67 scores, but only in some instances has this correlation been statistically significant [13,21], being weak [19-23] or absent [17,18] in many studies. No correlation between KS

histopathological stage and the percentage of Ki67-positive cells was found in another work [5], an observation that is supported by our results, which extend this lack of correlation to extracutaneous involvement. The absence of any direct or inverse correlation between $p27^{Kip1}$ and Ki67 expression in our cases is also consistent with these findings and provides further support for the association between $p27^{Kip1}$ down-regulation and KS progression.

In conclusion, down-regulation of $p27^{Kip1}$ expression, which may well be triggered by HHV8 infection, is probably implicated in KS progression through its various cutaneous histopathological stages and eventuaI extracutaneous spread.

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3. TERCER RESULTADO

OVER-EXPRESSION OF $P45^{SKP2}$ IN KAPOSI'S SARCOMA CORRELATES WITH HIGHER TUMOR STAGE AND EXTRACUTANEOUS INVOLVEMENT BUT IS NOT DIRECTLY RELATED TO $P27^{KIP1}$ DOWN-REGULATION.

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3.1. ABSTRACT

F-Box protein $p45^{SKP2}$ is the substrate-specific receptor of ubiquitin-protein ligase SCF/$p45^{SKP2}$ and is involved in the degradation of $p27^{Kip1}$ through the ubiquitin/proteasome pathway. In addition, $p45^{SKP2}$ facilitates proteolysis of other molecules related to the cell cycle, is frequently over-expressed in transformed cells, and induces S phase in quiescent cells. The aim of this study was to determine whether $p45^{SKP2}$ expression is altered in aggressive lesions of Kaposi's sarcoma and its relation to $p27^{KIP1}$down-regulation. We performed immunohistochemistry using antibodies directed to $p45^{SKP2}$, $p27^{KIP1}$, and Ki67 on paraffin blocks corresponding to 47 cases of Kaposi's sarcoma (8 macules, 10 plaques, 12 tumors, and 15 extracutaneous lesions). $p45^{SKP2}$ nuclear over-expression was present in all Kaposi's sarcoma stages, being significantly increased in skin tumors (mean ± 95% confidence interval: 39.2±18.8) and extracutaneous lesions (25.8±17.3) as compared with macules (18.9±8.2) and plaques (29.2±12.0; p=0.0199). On the other hand, Kaposi's sarcoma progression was associated with a decrease in $p27^{KIP1}$ expression and Ki67 immunoreactivity was independent of disease stage. No statistically significant differences were found in regard to patients´ sex and human immunodeficiency virus status and regression analysis failed to show a correlation among $p45^{SKP2}$, $p27^{KIP1}$ and Ki67 immunostaining scores. These findings suggest that $p45^{SKP2}$ is involved in Kaposi's sarcoma progression, not only by promoting the degradation of $p27^{KIP1}$ but also through other mechanisms still unknown.

KEY-WORDS

Kaposi's sarcoma, cell cycle, ubiquitin, proteasome, $p45^{SKP2}$, $p27^{KIP1}$, Ki67.

3.2. INTRODUCTION

Cell cycle progression requires the co-ordinated performance of a series of regulating molecules which orchestrate cycle transitions through either mitogenic or antiproliferative signals [1].

The two crucial mechanisms used by cells to control the protein levels required in each step of the cycle are protein synthesis and degradation. Disruption of these two mechanisms leads to abnormal cell proliferation and oncogenesis, particularly if the derangement results in loss of control at the G1-S transition.

One of the many cell-cycle regulating molecules is cyclin A, whose role as an S-phase propeller is the consequence of its ability to complex with its catalytic partner cyclin-dependent kinase 2 (CDK2). Subsequently, the latter acts as a modulator of the behavior of effector proteins such as the Kip/Cip family CDK inhibitors $p21^{CIP1}$ and $p27^{KIP1}$, transcription factor E2F-1, DNA replication and repair factor proliferating cell nuclear antigen (PCNA), and S phase kinase-asssociated proteins 1 and 2 ($p19^{SKP1}$ and $p45^{SKP2}$), among others [2].

In recent years, the mechanisms of protein degradation have attracted much attention and intense efforts have been made to elucidate the intricaces machinery involved in the ubiquitin proteasomal pathway, which plays a paramount role in the degradation of short-lived regulatory proteins involved in the cell cycle [3]. The two main steps followed by the ubiquitin proteasomal pathway are attachment of multiple ubiquitin molecules to a protein

substrate and degradation through the 26S-proteasome complex. Transference of ubiquitin to the substrate requires at least the collaboration of an ubiquitin-activating enzyme (E1) and an ubiquitin-conjugating enzyme (E2). Oftentimes, however, improvement of substrate recognition requires the co-operation of a third component termed ubiquitin–protein ligase (E3).

Recently, a novel class of E3 ubiquitin ligases named SKP1/CDC53(cullin)/F-box protein complex (SCF) has been described. This E3 class is involved in the degradation of several key cell-cycle regulatory proteins such as $p27^{KIP1}$, $p21^{CIP1}$ [4], E2F-1 [5], cyclin A [2], cyclin D [4], and cyclin E [6]. The SCF complex consists of the invariable components $p19^{SKP1}$, cullin-1 (Cul1 or CDC53) and regulator of cullins/RING box protein (Roc1/Rbx1) as well as a variable component known as F-box proteins. The latter include $p45^{SKP2}$, which by binding to $p19^{SKP1}$ is responsible for substrate specificity [7]. The SCF core complex is localized to the cytoplasm until $p45^{SKP2}$ is expressed in late G1 and moves the SCF/$p45^{SKP2}$ complex into the nucleus, where it promotes the ubiquitination of selected proteins. Nevertheless, a frequent splice variant named C-terminal variant ($p45^{SKP2}$-CTV) is found in the cytoplasm of various cell lines, in most cases associated with the usual form of $p45^{SKP2}$. Probably owing to this cytoplasmic mislocation, $P45^{SKP2}$-CTV is unable to achieve proper ubiquitination of the selected proteins [8]. The C terminal domain may contain a cytoplasmic retention domain that is dominant over the nuclear localization signal of $p45^{SKP2}$ failing to bind $p19^{SKP1}$ [9].

The fact that $p27^{KIP1}$ is rapidly degraded during late G1 phase in most cell types suggests that this cyclin-kinase inhibitor plays an important role in cell-cycle control, especially in

regard to the G–S transition. In various tumors decreased CDK inhibitor $p27^{KIP1}$ levels are associated with a poor prognosis [8-12]. The low levels of $p27^{KIP1}$ tumor suppressor may be due to either transcriptional and translational reduction, as in prostatic hyperplasia [11], or post-transcriptional protein degradation by the ubiquitin proteasomal pathway, as in numerous malignant neoplasms. Tumor-specific mutations in the $p27^{KIP1}$ gene, on the contrary, seem to be exceptional events.

Recent studies have demonstrated that $p27^{KIP1}$ degradation is mediated by the SCF/ $p45^{SKP2}$ complex and by ubiquitin-independent processing during progression from G1 to S phase [6]. $P45^{SKP2}$ specifically interacts with $p27^{KIP1}$ only when the CDK inhibitor is phosphorylated by cyclin E-CDK2, thereby promoting the ubiquitination and degradation of $p27^{KIP1}$. $P45^{SKP2}$ is frequently over-expressed in transformed cells, induces S phase in quiescent cells [13], and is a suspected proto-oncogene in human tumors. In fact, there are recent reports of increased levels of $p45^{SKP2}$ in association with reduced $p27^{KIP1}$ levels in epithelial neoplasms [13-16]. On the other hand, cyclin E expression is a periodic event that reaches maximal levels at the G1-S transition and cyclin E degradation is mediated by ubiquitin-dependent proteolysis. Both CDK2-associated and free forms of cyclin E appear to be targets for ubiquitination and rapid degradation, whereas binding of $p45^{SKP2}$ is one of the events involved in the proteolysis of free (but not of CDK2-associated) cyclin E [6]. Apart from their potential action as mediators of ubiquitin-dependent cyclin A proteolysis, $p19^{SKP1}$ and $p45^{SKP2}$ may also directly regulate the kinase activity of cyclin A-CDK2 [17].

Kaposi's sarcoma is an angiohyperplastic disease mediated by inflammatory cytokines and angiogenic growth factors in a setting of human herpesvirus type 8 infection. In advanced

stages Kaposi's sarcoma may behave as a multifocal neoplasm whose various lesions display monoclonality [18]. Immunosuppression is a triggering factor for Kaposi's sarcoma development, as demonstrated by the common occurrence of AIDS-associated Kaposi's sarcoma, which usually exhibits a much more aggressive behavior than classic Kaposi's sarcoma. In the skin, the organ primarily targeted by Kaposi's sarcoma, lesions are clinicopathologically classified into macules, plaques and tumors in agreement with their progression in severity [19]. In aggressive cases, usually of the AIDS-associated Kaposi's sarcoma form, the lesions may also involve extracutaneous locations.

We have previously demonstrated that decreased immunoreactivity for the cell-cycle regulator $p27^{KIP1}$ correlates with higher stage and extracutaneous involvement in Kaposi's sarcoma [20]. To the best of our knowledge, studies of $p45^{SKP2}$ expression have never been performed in Kaposi's sarcoma. Thus, the aim of this study was to determine whether $p45^{SKP2}$ expression is altered in aggressive Kaposi's sarcoma lesions and explore the possible relation of $p45^{SKP2}$ expression to $p27^{KIP1}$ down-regulation and other variables such as Ki67 expression, gender, and human immunodeficiency virus (HIV) infection.

3.3. MATERIALS AND METHODS

Human tissue samples

Cutaneous and extracutaneous Kaposi's sarcoma biopsy paraffin blocks were retrieved from the files of the Department of Pathology of Hospital Germans Trias i Pujol, Badalona, Barcelona, Spain. Paraffin blocks of extracutaneous cases of Kaposi's sarcoma were also provided by the Departments of Pathology of Hospital de la Santa Creu i Sant Pau and Hospital Prínceps d'Espanya, Barcelona, Spain. We evaluated 47 cases of Kaposi's sarcoma, of which 35 corresponded to AIDS-associated Kaposi's sarcoma and 17 to classic Kaposi's sarcoma. One of the 17 classic Kaposi's sarcoma cases followed an aggressive course and showed early extracutaneous involvement. The 47 Kaposi's sarcoma cases provided 32 skin biopsy specimens (8 macules, 10 plaques, and 12 tumors) and 15 biopsy specimens from extracutaneous locations (6 from lymph nodes, 4 from oral mucosa or conjunctiva, 2 from the gastrointestinal tract [including the one case of aggressive classic Kaposi's sarcoma], 1 from the larynx, 1 from the lung, and 1 from soft tissues).

The stage of cutaneous Kaposi's sarcoma cases was determined by histopathologic study of hematoxylin-eosin stained sections. Macular stage lesions consisted of a superficial or mid-dermal proliferation of collagen-dissecting jagged capillary vessels that disposed themselves around normal dermal structures. In some instances the newly formed vessels were confluent, but the spindle-cell component was always inconspicuous. A diagnosis of plaque stage Kaposi's sarcoma was made when the lesion consisted of a proliferation of malformed vascular channels that dissected collagen fibres and contained only isolated

spindle-shaped cells or small groups of them. Cases were classified as nodular or tumor phase Kaposi's sarcoma when the entire lesion or most of it showed a compact proliferation of spindle-shaped cells with an intersecting fascicle-like pattern alleviated only by some inflammatory cells, erythrocytes, or telangiectatic spaces. All tissue specimens had been fixed in neutral-buffered formalin and routinely processed.

Antibodies and immunohistochemical studies

Immunohistochemistry studies were performed using polyclonal anti-full-length SKP2 p45 antibody (Santa Cruz Biotechnology, Santa Cruz, California, USA, diluted 1:500 with phosphate-buffered saline (PBS)), anti-p27 protein mouse monoclonal antibody, clone 1B4, (Novocastra, Newcastle, UK, diluted 1:40 with phosphate-buffered saline (PBS)), and NCL-Ki67-MM1 mouse monoclonal antibody (Novocastra, diluted 1:50 with phosphate-buffered saline (PBS)). Five-micron-thick sections were deparaffinized, hydrated, immersed in buffered citrate and autoclaved. Afterwards, the sections were incubated for 30 minutes in rabbit serum. Incubations with primary antibodies were carried out for 22 hours at room temperature. Slides were washed and incubated with biotinylated rabbit anti-mouse Ig antibodies at a 1:700 dilution and then incubated in PBS/6% hydrogen peroxide for 15 minutes at room temperature before avidin-biotin peroxidase complex addition (Dakopatts, Glostrup, Denmark). The chromogen 3, 3'-diaminobenzidine tetrachloride (Serva, Heidelberg, Germany) was applied, and counter-staining was performed with Harris hematoxylin. A non-immune mouse serum was used as a negative control in this protocol.

Both elongated cells lining abnormal blood vessels and spindle-shaped cells unrelated to vessels were considered to be Kaposi's sarcoma neoplastic cells. Upon evaluating $p45^{SKP2}$ staining intensity, a sub-population of cells with strongly positive nuclei could be easily distinguished. The percentages of tumor cells showing $p45^{SKP2}$ positive nuclei, $p45^{SKP2}$ intensely positive nuclei, $p27^{KIP1}$ positive nuclei and Ki67 positive nuclei were independently assessed by two researchers (RMP and MTFF) in 500 tumor cells. In macule

and plaque stage lesions, where neoplastic cells were less abundant, twenty fields were evaluated. At least two hundred cells were counted in every case. The quotients (positive tumor cells/total number of tumor cells counted) were converted to percentages and rounded to the nearest integer. The arithmetic mean of both observers' scores was used for statistical evaluation. All cases in which inter-observer variation exceeded 10 per cent were jointly re-evaluated. In addition, the intensity of cytoplasmic stain for $p45^{SKP2}$ was jointly evaluated using the following score: 4, strong stain in at least 50% of cells; 3, strong stain in 25 to 50% of cells or moderate in more than 80% of cells; 2, strong stain in 5 to 25% of cells or moderate in 5 to 80% of cells; 1, moderate or strong stain in less than 5% of cells or weak in more than 5% of cells; 0, absent or weak stain in less than 5% of cells.

Statistical study

The statistical significance of differences observed between classic Kaposi's sarcoma and AIDS-associated Kaposi's sarcoma lesions and between plaque lesions and tumor lesions in regard to $p45^{SKP2}$, $p27^{KIP1}$, and Ki67 immunostaining scores, Kaposi's sarcoma clinical-epidemiological type, sex of patient, lesion location and clinicopathological type was determined using analysis of variance. When variances were not homogeneous or samples were not normally distributed, the Kruskal-Wallis test was used. In addition, $p27^{KIP1}$ expression was compared in the same groups using the cut-off described above and the Fisher's exact test for differences between proportions. Differences between groups were considered to be statistically significant when the *p* value was less than 0.05. The Pearson correlation coefficient with 95 per cent confidence limits was used to test the strength of association between $p27^{KIP1}$, $p45^{SKP2}$ and Ki67 expressions.

3.4. RESULTS

The results are summarized Tables 1, 2 and 3. We have found $p45^{SKP2}$ nuclear expression in all stages of Kaposi's sarcoma. The percentages of neoplastic cells expressing $p45^{SKP2}$ in their nuclei was significantly increased in skin tumors (mean ± 95% confidence interval: 39.2±18.8) and extracutaneous lesions (25.8±17.3) as compared with macules (18.9±8.2) and plaques (29.2±12.0; p=0.0199) (**Table 1**). The staining in some nuclei was distinctly intense, allowing separate quantification. The corresponding percentages were significantly increased in skin tumors (11.1±12.7) and extracutaneous lesions (7.4±5.8) with respect to macules (3.7±2.1) and plaques (4.8±3.7; p=0.0126). The differences between macules and plaques were not significant and, when lesions in both stages were put together (**Table 2**), the differences between groups became even more significant (p=0.0052). Interestingly, $p45^{SKP2}$ expression in extracutaneous Kaposi's sarcoma lesions was lower than in cutaneous tumors but higher than in plaques. As regards $p45^{SKP2}$ cytoplasmic stain, we observed a marked tendency toward an increase in the advanced stages (**Table 3**), but this trend did not reach statistical significance (p=0.0557 when macules and plaques were grouped together) owing to the non-continuous nature of the score we used. The results of the corresponding Kruskal-Wallis test are shown for comparison with nuclear staining scores.

We have found Kaposi's sarcoma progression to be associated with a decrease in $p27^{KIP1}$ expression. On the other hand, Ki67 expression levels were independent of the disease stage, as already demonstrated by a previous study of ours [20]. Regression analysis showed no statistical correlation between $p45^{SKP2}$ over-expression and loss of $p27^{KIP1}$. In

some Kaposi's sarcoma skin lesions a reduced expression of $p27^{KIP1}$ paralleled a high nuclear and cytoplasmic expression of $p45^{SKP2}$ (**Figure 1**), whereas in others high $p45^{SKP2}$ levels were associated with $p27^{KIP1}$ preservation (**Figure 2**). Similar findings regarding $p45^{SKP2}$ and $p27^{KIP1}$ expression were also observed in some extracutaneous Kaposi's sarcoma lesions (**Figure 3**), while in some aggressive lesions both molecules were poorly expressed. Moreover, whereas the maximum degree of $p27^{KIP1}$ down-regulation was observed in extracutaneous Kaposi's sarcoma lesions and then in cutaneous Kaposi's sarcoma tumors, the highest degree of $p45^{SKP2}$ expression corresponded to cutaneous Kaposi's sarcoma tumours and then to extracutaneous Kaposi's sarcoma lesions.

Immunohistochemical expression of $p45^{SKP2}$ was not correlated with Ki67 proliferation index, and no statistically significant differences were found in regard to patients´ sex and human immunodeficiency virus status (results not shown).

Table 1. Summary of mean scores of $p45^{SKP2}$, $p27^{KIP1}$, and Ki67 immunostaining in cutaneous and extracutaneous Kaposi's sarcoma lesions showing increasing severity and progression.

	Macules	**Plaques**	**Tumors**	**Extracutaneous**	***p***
$p45^{SKP2}$ nuclear	18.9±8.2	29.2±12.0	39.2±18.8	25.8±17.3	**0.0199**
$p45^{SKP2}$ intense nuclear	3.7±2.1	4.8±3.7	11.1±12.7	7.4±5.8	**0.0126**
$p45^{SKP2}$ cytoplasmic	0.6±0.7	0.8±0.6	1.8±1.0	1.8±1.4	0.0154
$p27^{KIP1}$ nuclear	86±12.8	74.3±26.5	55.4±26.0	45.3±24.3	**0.0012**
Ki67 nuclear	17.6±15.9	12.0±6.6	17.4±10.7	15.9±5.7	0.4124

The score is the percentage of positive neoplastic cells; results are given ± 95% confidence interval.

Table 2. Summary of mean scores of $p45^{SKP2}$, $p27^{KIP1}$, and Ki67 immunostaining in cutaneous and extracutaneous Kaposi's sarcoma lesions showing increasing severity and progression. Lesions corresponding to cutaneous macules and plaques are grouped together.

	Macules-plaques	**Tumors**	**Extracutaneous**	***p***
$p45^{SKP2}$ nuclear	24.6±11.5	39.2±18.8	25.8±17.3	**0.0304**
$p45^{SKP2}$ intense nuclear	4.3±3.1	11.1±12.7	7.4±5.8	**0.0052**
$p45^{SKP2}$cytoplasmic	0.7±0.8	1.8±1.0	1.8±1.4	0.0060
$p27^{KIP1}$ nuclear	79.5±21.8	55.4±26.0	45.3±24.3	**0.0012**
Ki67 nuclear	14.5±11.6	17.4±10.7	15.9±5.7	0.2930

The score is the percentage of positive neoplastic cells; results are given ± 95% confidence interval.

Table 3. Comparison of the number of cases with cytoplasmic $p45^{SKP2}$ immunostaining scores (0 to 4) in Kaposi's sarcoma lesions showing increasing severity and progression.

	Macules	**Plaques**	**Tumors**	**Extracutaneous**	**Total**
0	4	4	0	2	10
1	3	4	7	6	20
2	1	2	4	3	10
3	0	0	2	1	3
4	0	0	1	3	4
	8	10	14	15	47

$p45^{SKP2}$ cytoplasmic staining intensity was graded as follows: 4, strong stain in at least 50% of cells; 3, strong stain in 25 to 50% of cells or moderate in more than 80% of cells; 2, strong stain in 5 to 25% of cells or moderate in 5 to 80% of cells; 1, moderate or strong stain in less than 5% of cells or weak in more than 5% of cells; 0, absent or weak stain in less than 5% of cells.

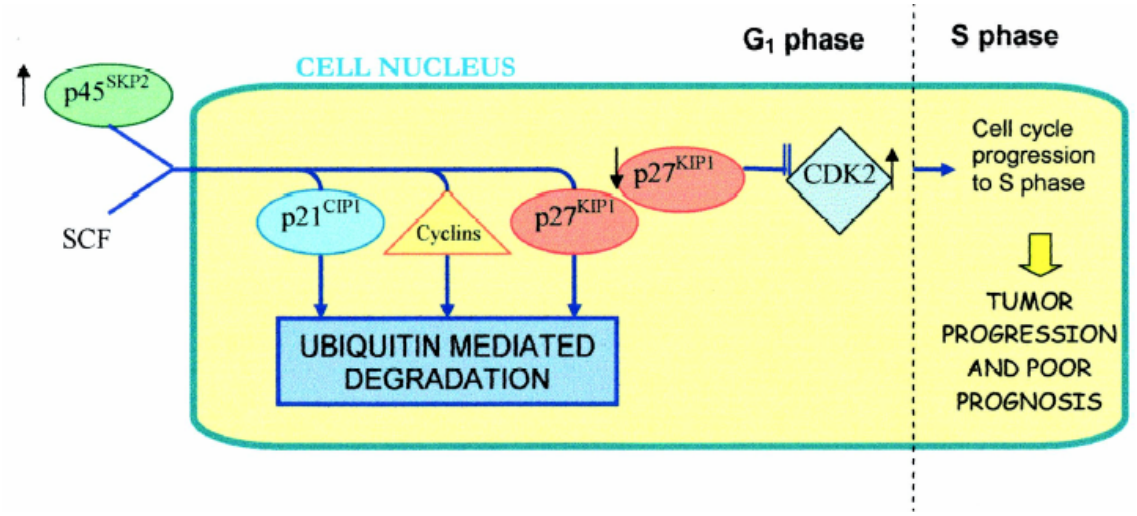

Diagram 1. General model of p45SKP2–p27KIP1–cyclin/CDK2 interaction. The SKP1/CDC53(cullin)/F-box protein complex (SCF) is involved in the degradation of several key cell-cycle regulatory proteins such as p27KIP1, p21CIP1, cyclin A, cyclin D, and cyclin E. The SCF core complex is localized to the cytoplasm until p45SKP2 is expressed in late G1 and moves the SCF/p45SKP2 complex into the nucleus, where it promotes the ubiquitination of selected proteins. p27KIP1 degradation is mediated by the SCF/p45SKP2 complex and by ubiquitin-independent processing during progression from G1 to S phase (6). The association between loss of p27KIP1 protein and uncontrolled proliferation of cancer cells is congruent with the function of p27KIP1 as a negative regulator of cyclins E and A, which in complex with CDK2 drive cells into the S-phase (15). P45SKP2 is frequently over-expressed in transformed cells, induces S phase in quiescent cells, and is a suspected proto-oncogene in human tumors. It has been postulated that low levels of p27KIP1 in aggressive human cancers may be caused by increased expression of p45SKP2 that targets p27KIP1 for ubiquitin-mediated degradation.

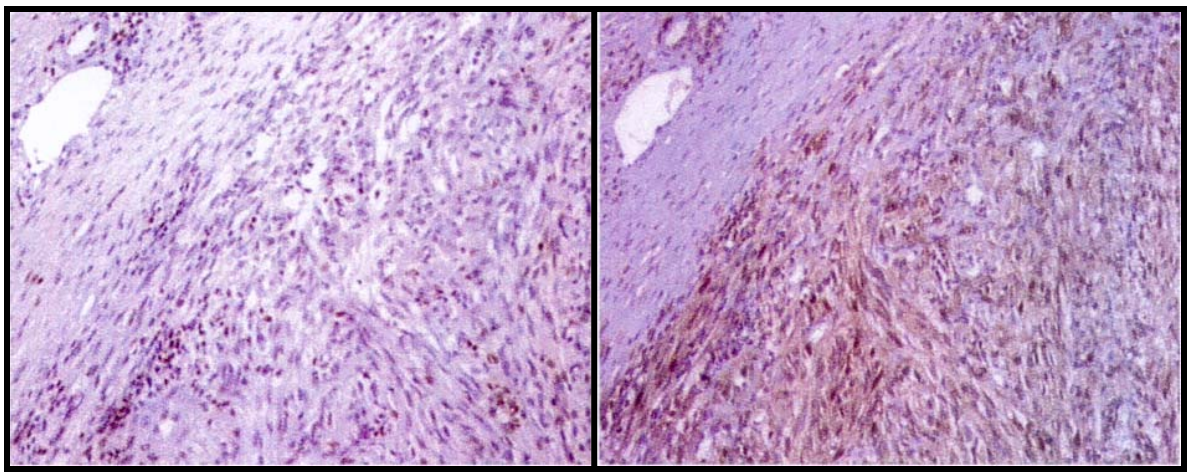

Fig. 1. Classic Kaposi's sarcoma tumor lesion with marked $p27^{KIP1}$ down-regulation (left) and $p45^{SKP2}$ over-expression in the same area (right). Slight cytoplasmic stain for $p45^{SKP2}$ is also present.

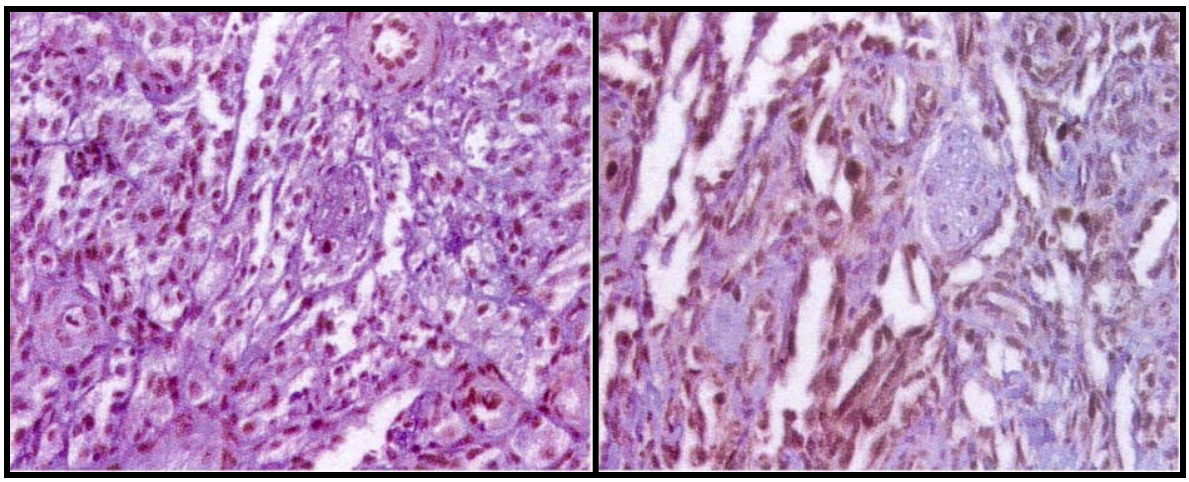

Fig. 2. Example of over-expression of both $p27^{KIP1}$ (left) and $p45^{SKP2}$ (right) in a classic Kaposi's sarcoma tumor lesion.

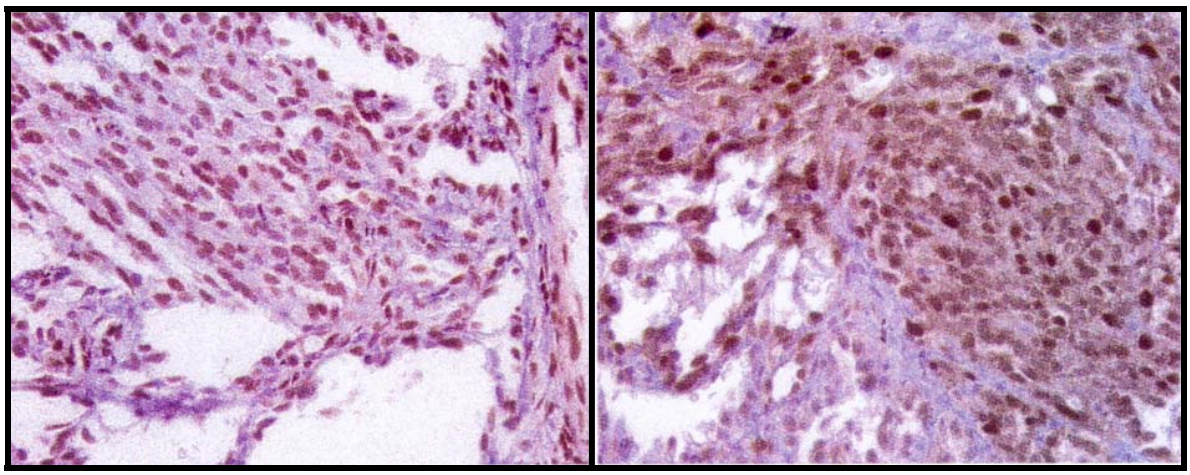

Fig. 3. Example of over-expression of $p27^{KIP1}$ (left) and $p45^{SKP2}$ (right) in a Kaposi's sarcoma extracutaneous lesion (lung). This case also displays strong cytoplasmic positivity and variable nuclear intensities of $p45^{SKP2}$ staining.

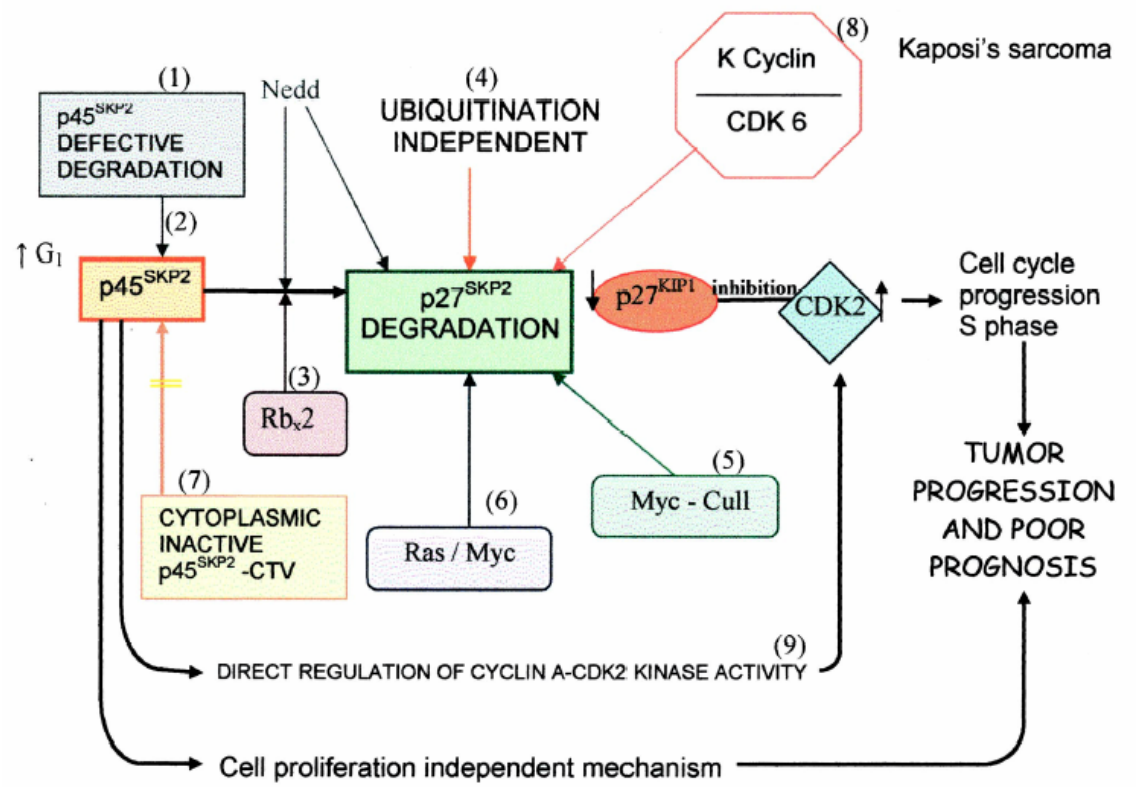

Diagram 2. Putative explanations for the lack of inverse correlations between p45SKP2 and p27KIP1 expressions in Kaposi's sarcoma. 1: Increased p27KIP1 degradation may be the result of a defective ubiquitination and degradation of p45SKP2 by either SCF/p45SKP2 action during the G0-G1 phase (23) or an alternative pathway independent of cell-cycle phase (24). 2: Nedd8 enhances SCF/p45SKP2 effect on p27KIP1 (27) and may regulate p27KIP1 turnover independently of p27KIP1 phosphorylation (28). 3: Rbx2 is also able to bind p45SKP2 and mediate p27KIP1 degradation (29). 4: p27KIP1 is also processed rapidly by an ubiquitination-independent mechanism that exhibits higher activity in the S phase than during the G0-G1 phase (30). 5: Increased p27KIP1 proteolysis in Kaposi's sarcoma may be caused by *Myc* oncogenic activation leading to Cul1 over-expression (25) or 6: by a proteolytic pathway activated by mitogens through *Ras* and *Myc* (26). 7: Increased expression of the inactive p45SKP2–CTV splice variant (8) might provide an explanation for nuclear p45SKP2 expression levels being lower in extracutaneous Kaposi's sarcoma lesions than in cutaneous Kaposi's sarcoma tumors with similar cytoplasmic p45SKP2 expression. 8: human herpesvirus type 8-encoded K cyclin, which is resistant to p27KIP1 CDK inhibition, would facilitate p27KIP1 phosphorylation and down-regulation, thus enabling activation of endogenous cyclin/CDK2 complexes (31, 32). 9: p45SKP2 may also directly regulate cyclinA-CDK2 kinase activity (17) and contribute to the malignant phenotype without affecting proliferation (14).

3.5. DISCUSSION

Numerous studies have shown that reduced levels of $p27^{KIP1}$ protein, an inhibitor of cyclin-dependent kinases, are associated with a more aggressive course and a poorer prognosis in a large variety of carcinomas. Moreover, in the case of colon [10,15], breast [12] and prostate cancers [11] low $p27^{KIP1}$ expression provides independent prognostic information. The association between loss of $p27^{KIP1}$ protein and uncontrolled proliferation of cancer cells is congruent with the function of $p27^{KIP1}$ as a negative regulator of cyclins E and A, which in complex with their catalytic partner CDK2 drive cells into the S-phase [15]. In the normal cell cycle, the G0/G1 phase is characterised by high $p27^{KIP1}$ levels and low $p45^{SKP2}$ levels. Subsequently, during the S-phase, $p45^{SKP2}$ levels increase and $p27^{KIP1}$ is rapidly degraded, thus allowing the promotion of cell proliferation by the conjoint action of cyclin E/CDK2 and cyclin A/CDK2 [15]. $P27^{KIP1}$ appears to belong to a recently recognized class of tumor suppressors in which reduced protein expression is usually not caused by genetic change. Recent studies have identified the machinery involved in $p27^{KIP1}$degradation as an SCF type ubiquitin ligase complex that contains $p45^{SKP2}$ as the specific substrate-recognition unit. Levels of $p45^{SKP2}$ are rate-limiting for the degradation of $p27^{KIP1}$ [21], and it has been postulated that low levels of $p27^{KIP1}$ in aggressive human cancers may be caused by increased expression of $p45^{SKP2}$ that targets $p27^{KIP1}$ for ubiquitin-mediated degradation. Levels of $p45^{SKP2}$ expression correlate directly with malignancy grade and inversely with $p27^{KIP1}$ levels in human lymphomas [22], colorectal carcinomas [15] and oral squamous cell carcinomas [14,16]. In the normal cell cycle, $p45^{SKP2}$ levels are very low in the G0/G1 phase, increase in the S-phase, and decline afterwards [2]. High levels of $p45^{SKP2}$ are not due just to increased proliferation, despite the

direct correlation observed in lymphomas [22], inasmuch as the percentage of cells expressing high levels of $p45^{SKP2}$ in colorectal carcinoma greatly exceeds the percentage of cells expected to be in the S-phase in a randomly dividing population [15]. Increased $p45^{SKP2}$ protein levels do not always correlate with increased cell proliferation (as assayed by Ki67 staining), which suggests that $p45^{SKP2}$ alterations may contribute to the malignant phenotype without affecting proliferation [14]. Our results indicate that the aforementioned observations also apply to Kaposi's sarcoma, although the picture seems to be somewhat more complicated in this neoplasm. Specifically, the findings in need of alternative explanatory hypotheses in Kaposi's sarcoma are the lack of an inverse correlation between $p27^{KIP1}$ and $p45^{SKP2}$ levels and the apparent paradox of $p45^{SKP2}$ expression, which in extracutaneous Kaposi's sarcoma lesions happens to be lower than in cutaneous Kaposi's sarcoma tumors but higher than in Kaposi's sarcoma macules/plaques.

Increased $p27^{KIP1}$ degradation may be the result of a defective ubiquitination and degradation of $p45^{SKP2}$ by either SCF/ $p45^{SKP2}$ action during the G0-G1 phase [23] or an alternative pathway independent of cell-cycle phase [24]. In addition to $p45^{SKP2}$ over-expression, increased $p27^{KIP1}$ proteolysis in Kaposi's sarcoma and other neoplasms may be caused by *Myc* oncogenic activation leading to Cull over-expression [25]. $p27^{KIP1}$ control may be also achieved by a second proteolytic pathway that is activated by mitogens through *Ras* and *Myc* and is operative during the G1 phase [26].

Nedd8, an ubiquitin-like protein expressed in proliferating cells, acts on Cul1, enhances SCF/ $p45^{SKP2}$ effect on $p27^{KIP1}$ [27], and may regulate $p27^{KIP1}$ turnover independently of $p27^{KIP1}$ phosphorylation [28]. Also able to bind $p45^{SKP2}$ and mediate $p27^{KIP1}$ degradation is

Rbx2, which is the product of the sensitive-to-apoptosis gene **(*SAG*)** and the second member of the RING box protein family [29]. In parallel with its ubiquitin-dependent degradation, $p27^{KIP1}$ is processed rapidly by an ubiquitination-independent mechanism that exhibits higher activity in the S phase than during the G0-G1 phase [30].

On the other hand, increased expression of a $p45^{SKP2}$ splice variant that localizes to the cytoplasm and fails to direct cyclin D1 (and supposedly $p27^{KIP1}$) ubiquitination and degradation [8] might provide an explanation for our finding that nuclear $p45^{SKP2}$ expression levels are lower in extracutaneous Kaposi's sarcoma lesions than in cutaneous Kaposi's sarcoma tumours with similar cytoplasmic $p45^{SKP2}$ expression levels.

The fact that Kaposi's sarcoma is related to human herpesvirus type 8 infection suggests a plausible explanation for the lack of an inverse correlation between $p27^{KIP1}$ and $p45^{SKP2}$ expression levels in this neoplasm. Specifically, human herpesvirus type 8 -encoded K cyclin, which is resistant to the actions of $p16^{INK4A}$, $p21^{CIP1}$ and $p27^{KIP1}$ CDK inhibitors, would bypass a $p27^{KIP1}$-imposed G1 arrest by facilitating $p27^{KIP1}$ phosphorylation and down-regulation and thus enabling activation of endogenous cyclin/CDK2 complexes [31,32]. Indeed, the occurrence of a $p27^{KIP1}$-phosphorylating CDK6 complex in cell lines derived from primary effusion lymphoma and Kaposi's sarcoma may well indicate that virally induced $p27^{KIP1}$ degradation takes place in human herpesvirus type 8 -related tumours [32].

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