Role of mitogen-activated protein kinase (MAPK) in the sporadic renal cell carcinoma

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Abstract

Context: Only on the basis of the involvement of the vhl suppressor gene in the cases of renal cell carcinomas (RCC), the involvement of the signaling pathway between the pVHL and the hypoxia inducible factor 1 alpha (HIF-1\(\alpha\)) has been evaluated because of the need to find new diagnostic and prognostic response to drugs markers.

Evidence synthesis: The overexpression of HIF-1\(\alpha\) confers better prognosis in clear cell type RCC (ccRCC). Furthermore, HIF-1\(\alpha\) regulates other genes, specifically that of the carbon anhydrase IX (CA-IX), whose overexpression is practically only one of the ccRCC and its determination is useful for this subtype. However, the involvement of the CA-IX has not been demonstrated in the prognosis or in the response to immunomodulators or antiangiogenics. Therefore, it is necessary to make a global evaluation of all this pathway: pVHL→HIF-1\(\alpha\)→CA-IX, and even the analysis of other proteins and signaling pathways that also control the HIF-1\(\alpha\) activity. In the latter case, the MAPK are critical in the HIF-1\(\alpha\) activation, there being evidence on the experimental level of the control on its activity. Although the role of the MAPK in the phenomena of resistance to conventional chemotherapy and radiotherapy has been demonstrated, it has not been demonstrated in response to sorafenib, an important piece of information if we consider that it is an inhibitor of several protein kinases. Recently, it has been observed that the MAPK may be involved in the responses to different therapies, included those based on tyrosine kinase inhibitors.

Conclusions: The confirmation of these data would suppose an explanation of the variation observed between patients who, with the same functional alteration of the vhl gene, have a different biological, clinical behavior and better selection of non-surgical therapies.

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Papel de las proteínas quinasas activadas por mitógenos (MAPK) en el carcinoma de células renales esporádico

Resumen

Contexto: Últimamente, basándose en la implicación del gen supresor vhl en los casos de carcinoma de células renales (CCR), se ha evaluado la implicación de la ruta de señalización entre pVHL y el Factor Inducible por Hipoxia 1 alfa (HIF-1α), ante la necesidad de encontrar nuevos marcadores diagnósticos, pronósticos y de respuesta a fármacos.

Síntesis de evidencia: La sobreexpresión de HIF-1α confiere mejor pronóstico en pacientes afectos de CCR de tipo células claras (ccRCC). Además HIF-1α regula otros genes, concretamente el de la anhidrasa carbónica IX (CA-IX), cuya sobreexpresión es prácticamente exclusiva de los ccRCC y su determinación útil para el diagnóstico de este subtipo. Sin embargo, no se ha demostrado la implicación de CA-IX ni en el pronóstico, ni en la respuesta a inmunomoduladores o antiangiogénicos. Ello hace necesario la evaluación global de toda esta ruta: pVHL → HIF-1α → CA-IX, e incluso el análisis de otras proteínas y vías de señalización que también controlan la actividad de HIF-1α. En este último caso, las MAPK, son críticas en la activación de HIF-1α, existiendo evidencias a nivel experimental del control sobre su actividad, aunque no se ha establecido su papel clínico como biomarcador. Si bien está demostrado el papel de las MAPK en los fenómenos de resistencia a quimio y radioterapia convencional, no lo está en la respuesta a sorafenib, dato llamativo si tenemos en cuenta que es inhibidor de varias proteínas quinasas. Recientemente se ha observado que las MAPK pueden estar implicadas en la respuesta a distintas terapias, incluidas las basadas en inhibidores de tirosin quinasa.

Conclusiones: La confirmación de estos datos, supondrá una explicación a la variación observada entre pacientes, que con una misma alteración funcional del gen vhl, presentan un distinto comportamiento biológico y clínico, y a una mejor selección de terapias no quirúrgicas.

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Context
Renal tumors account for approximately 3% of all neoplasias, renal cell carcinoma (RCC) being the most common malignant tumor in the adult kidney.

Both generally and in our environment, the incidence of RCC has increased about 126% since 1950, producing a progressive annual increase in incidence rates of 2.3–4.3%, mainly due to increased use of image techniques. However, in parallel, mortality has increased by 36.5% annually, so the incidental detection alone does not fully explain this increased incidence, which leads us to believe that other factors such as environmental, dietary, or genetic seem to be involved as well in the growing diagnosis of this tumor. Within the RCC, the most common is the clear cell carcinoma (ccRCC), which represents about 70%.

The RCC can occur in the context of hereditary diseases or sporadically, classified, thus, in family kidney tumors and sporadic tumors. The sporadic form accounts for 96% of the cases, hence the great interest in understanding the molecular mechanisms that start its formation. Among the hereditary forms, the best characterized one is that associated with the Von Hippel Lindau syndrome, which is characterized because the affected individuals are at risk of developing tumors in different organs, including the kidneys, the cerebellum, the spinal column, the inner ear, the adrenal glands, and the pancreas. In this syndrome, genetic alterations particularly affecting chromosome 3 have been found. Specifically, in recent years, the vhl gene has been identified, located on the short arm of chromosome 3 (3p), classed as a tumor suppressor gene and establishing a close relation between this gene and the Von Hippel Lindau syndrome, in such a way that this is altered in more than 70% of the cases. However, the involvement of this gene has also been verified in the occurrence of sporadic RCC cases, a remarkable relation between alterations in the vhl gene with the RCC being found, mainly in clear cell type, something also ratified by previous studies by our group.

In recent years, and given that the diagnostic criteria based solely on the histological architecture are insufficient, we are trying to perform a molecular characterization of the RCC to establish new tumor markers useful both in the histological diagnosis and in the prognostic prediction and drug response that are now being developed. In this regard, and based on the aforementioned involvement of the vhl suppressor gene in sporadic RCC cases, we evaluated the involvement of the signaling pathway between VHL and the hypoxia-inducible factor 1 alpha (HIF-1α) in the RCC.

Evidence synthesis
pVHL pathway → HIF-1α → CA-IX

The vhl gene encodes a protein (pVHL) binding to the HIF-1α in normoxic conditions, favoring its degradation, mediated by ubiquitination and by a mechanism dependent on prolyl hydroxylases (hydroxylation of prolines 402 and 564). Herein lies part of the suppressor function of the vhl gene. The absence of a normal function of the pVHL (either by mutation or by hypermethylation of the gene) creates a situation similar to hypoxia, so the HIF-1α is not
degraded, resulting in high levels of the HIF-1α that are characteristic of the RCC. In addition, the HIF-1α is also a transcription factor that induces the activation of certain genes, whose proteic product increases oxygen availability by activation of angiogenic factors (mainly VEGF, PDGF, and erythropoietin) or regulates metabolic adaptation when the environmental oxygen is limited. By contrast, in the presence of normal activity of pVHL and under normal oxygen environmental conditions, the HIF-1α is degraded not exercising its function. Overproduction of angiogenic factors due to increased HIF-1α may explain the hypervascular nature of the RCC and the possible stimulus to the tumor development and growth.6-10 As described in other works, overexpression of the HIF-1α could confer better prognosis in patients with ccRCC.8,9 Moreover, the HIF-1α also regulates other genes; among which carbonic anhydrases (CA) are particularly relevant, specifically the gene that produces the protein CA-IX. This, except in the gastric mucosa, is expressed mainly in tumor tissues, including the RCC, and it is also induced by hypoxic conditions.11,12 Overexpression of the CA-IX is almost exclusive to ccRCC,13 so its measurement is useful in the diagnosis of this cell subtype. When overexpressed in other subtypes, there is the possibility that it is the case of false positives or RCC with mixed component. The expression of the CA-IX can be determined by immunohistochemistry (IHC) or direct methods such as Western Blot (WB). Studies so far have not demonstrated the involvement of the CA-IX in the prognosis of RCC – in terms of survival or tumor progression – or in the response to certain immunomodulatory or anti-angiogenic therapies, objectifying only in cases of metastatic RCCs.14-17 the usefulness of the CA-IX being more directed to the differential diagnosis of the histologic subtypes of the RCC.16 Therefore, further studies involving a comprehensive assessment of all this signaling pathway \(\text{pVHL} \rightarrow \text{HIF-1α} \rightarrow \text{CA-IX}\) are needed, and even the analysis of other proteins and signaling pathways that, like mitogen-activated protein kinases (MAPK) or ATM, monitors the activity of the HIF-1α.

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Along with the mechanism based on proline hydroxylase-VHL, the full functionality of the HIF-1α is controlled by different mechanisms involving acetylation, as described with the lysine 53218 and phosphorylation. In the latter case, the MAPK appear to be critical in the activation of the HIF-1α. Thus, it has been reported that the ERK1/2 or p38 MAPK are able to phosphorylate HIF-1α in vitro19,20 and how the inhibition of these two MAPK is capable of blocking the expression of reporter genes of the HIF-1α activity. In fact, it is known how the re-stabilization of the HIF-1α, through nitric oxide or chromium, is mediated by the MAPK.21 Even it has been described how another MAPK, the ERK5, can control the HIF-1α expression levels at least in endothelial cells, promoting their degradation.22 Recently, it has also been shown how the inhibition of various MAPKs blocks the growth of cell models derived from ccRCC.23 In short, there is noticeable evidence to suggest that the MAPK signaling pathways may be critical in the biological effects of the HIF-1α.

The MAPK are a family of serine/threonine kinases activated by growth and stress factors. These proteins play a key role in intracellular signal transduction, allowing the cell to integrate different extracellular stimuli. Thus, the MAPKs regulate processes such as mitosis, changes in patterns of gene expression, movement, metabolism, programmed cell death allowing cells to survive, proliferate, induce apoptosis, interact with multiple cell types, etc. All these processes are involved in the proper development of the organism, as well as in its homeostasis, with implications for cancer and its therapy.23 From the phylogenetic point of view, the MAPKs are part of the CMGC group of protein kinases, of which the cyclin-dependent kinases (CDK), glycogen synthase kinases, and CDK-like kinases are also part.26 Each MAPK subfamily consists of a signaling module of three evolutionarily conserved kinases that are activated sequentially. Thus, the MAPK kinase kinase (MAPKKK or MEKK) activates the MAPK kinase (MAPKK or MEK), which in turn activates the MAPK through phosphorylation. MAPKKKs are proteins often activated by phosphorylation or by direct interaction with GTP-binding proteins (such as the Ras/Rho family) in response to extracellular stimuli.27 Activation of these MAPKKs causes activation in serine or threonine residues of the MAPKs, and their activation in turn stimulates the phosphorylation and activation of the MAPKs by dual phosphorylation on threonine and tyrosine residues (motif Thr-X-Tyr) located in the activation loop of kinase subdomain VIII.28 Once active, they phosphorylate their substrates on serine or threonine residues adjacent to proline. The signal transmission fidelity will be determined by the sequential activation of these proteins through the participation of specific adapter proteins, whose function is to organize the proteins of the pathway allowing for a coordinated activation. The duration and intensity with which the pathway is activated will depend on the subcellular compartmentalization of the various molecules involved in the pathway (for example PEA-15 that retains ERK1/2 in the cytoplasm) and the activation of phosphatases that silence the route when necessary. In response to these stimuli, three MAPK protein subfamilies can be activated: ERK, SAPK/JNK, and p38 MAPK.

From everything mentioned above, it seems logical to believe that, in addition to the functionality of the pVHL, which is undoubtedly the key element in the transformation process of the ccRCC, the MAPKs may play an important role. In this regard, it is noteworthy that in 1995, Oka et al.29 described how in a small series of patients with ccRCC constitutive activation of ERK1/2 was detected. However, this study is based on a complex methodology (in vitro kinase assays) susceptible to multiple devices. More recently, the use of p38 as a potential biomarker of ccRCC, which correlates as well with Fuhrman grade,30 has been proposed. Other studies have shown a significantly high expression of MAPKK1 and ERK2 in ccRCC relative to controls, suggesting that this pathway may have therapeutic value against ccRCC.24 In order to test this hypothesis, we used the Anthrax lethal toxin (LeTx) as an inhibitor of the multiple MAPK signaling pathways. LeTx is an exotoxin, which partly, binds the \(\text{NH}_2\)-terminal of MKK1, MKK2, MKK3, MKK4, MKK6, and MKK7, but not MKK5, resulting in a loss of function of the MKK kinase.24
Conclusions

Although there is great evidence at the experimental level on the role of MAPK-mediated signaling in controlling the activity of the HIF-1α, its clinical role as a biomarker in ccRCC has not been established. Furthermore, although the role of the MAPK in the phenomena of resistance to conventional chemotherapy and radiotherapy has been shown, it has not in resistance to sorafenib, a treatment which currently has the RCC as one of its indications. This finding is striking if we take into consideration that sorafenib, although it is an inhibitor of several protein kinases, was initially considered as an inhibitor of Raf, which is the MAPKK of the signaling pathway of ERK1/2. Moreover, it has recently been observed that the MAPK may be involved in the activity of different cancer therapies, including those based on tyrosine kinase inhibitors as in the case of imatinib mesylate.

Confirmation of all these data could mean further explanation for the variation observed among patients who, with the same functional alteration of the vhl gene, have a different biological and clinical behavior. In addition, being able to determine their relation with the response to the new drugs, such as sorafenib, will contribute to a better selection of non-surgical therapies administered to these patients.

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Conflict of interest

The authors declare that they have no conflict of interest.

References


