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K-RAS-CALMODULIN INTERACTION IN DUCTAL ADENOCARCINOMAS: BRIEF REVIEW

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ABSTRACTS

Oncogenic K-Ras has been implicated in most adenocarcinomas and the tumors initiated by K-Ras are known to be more aggressive as compared to the tumors initiated by other isoforms belonging to the Ras superfamily, like N-Ras and H-Ras. In spite of the essential role played by the Ras proteins in cellular signaling, their signaling has not been fully understood; Studying Ras signaling, especially for K-Ras-induced ductal adenocarcinomas, is crucial in order to develop efficient strategies to inhibit or suppress their role in tumor initiation and progression. Of the many different molecules and pathways that K-Ras triggers, calmodulin is an important one, which also has the capacity to modulate many different signaling processes, including cell proliferation pathways like the canonical and non-canonical Wnt signaling pathway and the PI3K/Akt pathway. Here we discuss in brief, the interaction of Calmodulin with K-Ras and understand how this interaction can affect the normal cellular signaling, particularly the canonical and non-canonical Wnt signaling pathway, to lead to the initiation of ductal adenocarcinomas. Interestingly, it has been seen in clinically relevant studies that Ca2+ and calmodulin levels in ductal adenocarcinomas like pancreatic adenocarcinomas, lung adenocarcinomas and colorectal carcinomas, are relatively higher; So, the probability of a functionally important interaction between K-Ras and Calmodulin needs to be intricately investigated in reference to ductal adenocarcinomas. A detailed study in this regard would help develop strategies of blocking the K-Ras-calmodulin interaction and could be of immense therapeutic value in the treatment of this dreaded category of cancers – the ductal adenocarcinomas.

KEYWORDS: K-Ras, Calmodulin, Ductal Adenocarcinoma, Wnt pathway.

INTRODUCTION

The presence of oncogenic K-Ras has been observed in most cancers and the tumors initiated by K-Ras are known to be more aggressive as compared to the tumors initiated by other isoforms belonging to the Ras superfamily, like N-Ras and H-Ras. In spite of the essential role played by the Ras proteins in cellular signaling, their signaling has not been fully understood.[1] Ras triggers many cellular signaling pathways in different cancers, leading to cell survival and proliferation, migration, autophagy, etc.[2] Ras-stimulated signaling includes the MAPK pathway, the PI3K/AKT pathway and RalA/B which may be triggered differentially in different types of cells, further increasing the complexities of Ras-mediated signaling in different cells. Such functional complexity also leads to the build-up of drug-resistance that is also expected to be mediated via Ras molecules. To add to this functional heterogeneity, different variants of K-Ras have different capacities. Even in oncogenic K-Ras, specifically its splice variant, K-Ras4B, is the only variant known to induce the tumorigenesis of adenocarcinomas.[3] K-Ras4B has the ability to specifically interact with Calmodulin, which is another extremely important molecule in cellular signaling, in that it also has the capacity to temporally regulate many different signaling processes, including cell proliferation pathways like the canonical and non-canonical Wnt signaling pathway and the PI3K/Akt pathway. Considering this information, it is clear that the signaling pathway being triggered by K-Ras, the extent to which it is triggered and how it affects other types of signaling molecules in its surrounding microenvironment, is mind-boggling.

This review intends to focus on a very small part of this complex signaling network – the interaction of Calmodulin with K-Ras – and understand how this interaction can affect the normal signaling of a cell, to lead to the initiation of ductal adenocarcinomas. Some literature points at the regulating effect of K-Ras-Calmodulin on canonical and non-canonical Wnt signaling in ductal adenocarcinomas. Interestingly, it has been seen in clinically relevant studies that Ca^{2+} and calmodulin levels in ductal adenocarcinomas like pancreatic ductal adenocarcinomas (PDAC), colorectal

adenocarcinomas (CRC) and lung adenocarcinomas (LUAC), are relatively higher. So, the probability of a functionally important interaction between K-Ras and Ca^{2+}/Calmodulin needs to be intricately investigated in reference to ductal adenocarcinomas. Studying this interaction could help in developing efficient therapeutic strategies to block their effect on tumor initiation and progression; it is especially true for K-Ras-induced ductal adenocarcinomas.

K-Ras in different ductal adenocarcinomas: K-Ras or V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog, is a gene first isolated from Kirsten murine sarcoma virus (Hence the name 'K'-Ras), and is encoded by the KRAS gene in humans. K-Ras protein (KRAS gene located at chromosome 12p12.1) is a GTPase, that is a member of the canonical Ras superfamily of proteins that also includes H-Ras (HRAS gene located at chromosome 11p15.5) and N-Ras (NRAS gene located at chromosome 1p13.1).[4] The Ras molecules are early acting GTPases in many signaling pathways, as they are usually tethered to or situated close to the cell membrane, in the vicinity of EGF receptors, since EGF binding triggers Ras molecules. It has been known for more than three decades now, that specific mutations in these three Ras family genes lead to constitutive activation of Ras in a wide variety of human cancers. According to a review published by Bos et al.., of all the Ras-associated mutations, which amount to more than 30% of all human cancers, K-Ras is known to be the most mutated member.[5] Baines et al., in their review mention that KRAS mutations comprise 86% of all Ras mutations.[6] Another review mentions that Ras mutations appear in 90% of pancreatic, 45% of colon and 35% of lung cancers[7] and K-Ras is preferentially over-expressed in many adenocarcinomas as that of the pancreas, lungs, colon and intestine; moreover, the single nucleotide substitution that leads to K-Ras mutation, is associated with worse prognosis and worse overall survival of pancreatic and colon adenocarcinomas respectively.[8,9]

In their review on pancreatic adenocarcinomas, Mann et al. mention K-Ras is constitutively activated in 90% of pancreatic cancer cases. In pancreatic cancers that lack KRAS mutations, signaling molecules upstream or downstream of K-Ras, like EGFR or B-Raf respectively, are mutated. In pancreatic adenocarcinomas, Ras triggers many signaling pathways which lead to complexity related with cross-activation of different molecules of the signaling network. This, consequently makes it extremely difficult to have effective targeted therapies against erratic Ras signaling in pancreatic cancer, for which combinatorial therapies are now being explored for better results.[2]

According to a study done by Lüchtenborg et al., in sporadic colorectal carcinomas too, K-Ras mutation is significant, occurring with a frequency of 37%, which is in conjunction with the earlier reported frequencies of 30 to 60%. It has been reported that early to intermediate stages of the majority of colorectal tumors have aberrations in K-Ras which occur in a co-dependent manner with the Wnt signaling.[10]

Bienz and Clevers et al.[11] suggest in their review that, oncogenic activation of K-Ras are frequently observed in colorectal tumors, but cannot initiate tumorigenesis alone. In humans, oncogenic K-Ras apparently contributes to tumor progression relatively early, during the transition from moderate to late adenomas. There appear to be some differences between mice and humans in the factors that are essential for tumor progression. An explanation for this could be that β-catenin activation leads to tumors predominantly in the small intestine of the mouse, but in the colorectum of humans. Furthermore, some apparent differences may reflect the different life and survival spans — on the order of months in mice, but decades in humans.

Oncogenic mutations in KRAS are observed in approximately one-third of cases of lung adenocarcinoma (LUAC) and are strongly associated with smoking. Oncogenic K-Ras leads to lung cancers by interacting with chronic inflammatory pathways [12]. In their review, Baines et al. state that, lung cancer is the leading cause of cancer-related deaths in the United States, and that K-Ras had the highest mutation frequency in lung cancer. They also state that K-Ras is the most significant target for new therapies for these deadly cancers.[6]

Wnt signaling in different cancers: The wnt1 gene was identified in 1982 by Nusse and Varmus in virally induced breast tumors.[13] Since then, owing to extensive research, a complete family of 19 Wnt proteins is known, which play a very important role in development and growth during embryonic stages and development of cancer in adult cells too. The Wnt proteins act as ligands for the transmembrane receptors called Frizzled (Fzd), and control many aspects of cellular progression and maintenance by triggering downstream signaling. When ligands like Wnt1 attach to Fzd1, Fzd4 or Fzd10, canonical Wnt signaling is triggered, which leads to upregulation of β-catenin and its nuclear translocation. Here, β-catenin binds to, and triggers activation of transcription factors like TCF, LEF, which in turn, upregulate gene transcription of proteins involved in cell survival and proliferation.[14]

The non-canonical Wnt pathway or the Wnt/Ca^{2+} signaling pathway functionally antagonizes canonical Wnt or the Wnt/β-catenin pathway. In the Wnt/Ca^{2+} pathway, ligands like Wnt5a bind to particularly Fzd8, and activate Ca^{2+}/Calmodulin protein kinase II (CamKii). This activation results in the inhibition of gene transcription, thereby regulating cell survival and cell growth.[14]

The cellular control imposed by the Wnt pathway is of utmost importance in understanding more about

colorectal cancers as a mutation in any genes related to Wnt signaling can lead to erroneous β-catenin regulation and development of colorectal cancers.[10]

Ca2+/Calmodulin in relation to cancers: Calmodulin (CaM), which also acts as the sensor for Ca^{2+}, is a small Ca^{2+}-binding, dumbbell-shaped molecule of approximately 148 amino acids, abundantly found in all mammalian cells. CaM has two domains attached by a central alpha helix and two helix-loop-helix regions per domain, that have identical Ca^{2+} binding sites; thus, each CaM binds four Ca^{2+} ions, two on each side. CaM plays a significant role in regulating various normal cellular processes, like cell growth, differentiation, survival and proliferation, or cell motility and also in the tumor-associated signaling by interacting with different CaM-binding proteins. In some cells, CaM downregulates MAPK signaling and up-regulates the PI3K/Akt signaling via Ras[15] A critical capacity of Ca2+/CaM is the ability to regulate the canonical and non-canonical Wnt pathways, by interacting with oncogenic K-Ras. Villalonga et al. have shown through their work that Calmodulin (CaM) binds preferentially to GTP-bound K-Ras4B in a Ca^{2+} dependent manner.[15] This interaction reduces the amount of CaM available for interaction with CaM Kinase II (CaMKii), which is a downstream effector of Wnt/Ca^{2+} or Non-canonical Wnt pathway.[16] Thus, it would be easier to understand that given that the non-canonical pathway is downregulated, more canonical Wnt signaling would take place, and more β-catenin would be available to trigger the expression of genes required for cellular proliferation, like c-myc, Cyclin-D, etc. If CaMKii binds to CaM, the non-canonical Wnt signaling would be maintained at levels where the β-catenin would be controlled appropriately such that the expression of cellular proliferation genes is not triggered. Wang et al. have shown through their work that oncogenic K-Ras can bind to CaM and via a series of activations, trigger the canonical Wnt signaling pathway. In this case, it is necessary that CaM interacts more with unphosphorylated K-Ras, instead of CaMKii.[17] The other way that Ca^{2+}/Cam can regulate the cellular progression is by Ca^{2+}-triggered activation of Phospho-kinase C (PKC) which phosphorylates K-Ras, thereby inhibiting its ability to interact with CaM and trigger canonical Wnt signaling.[18] Also, CamK11 can antagonize β-catenin/TCF signaling via TAK1 and NLK, thereby controlling cell proliferation-associated signaling.[19]

In their review, highlighting importance of Calmodulin in K-Ras-induced adenocarcinomas, Nussinov et al. have suggested that CaM has the ability to control and manipulate cellular signaling in K-Ras-induced adenocarcinomas. They suggest that Calmodulin/Ca^{2+} bind K-Ras4B and modulate MAPK and PI3K/Akt signaling in ductal adenocarcinomas. K-Ras4B-CaM interaction could be responsible for the increase in proliferation and migration in pancreatic ductal adenocarcinomas, colorectal carcinomas, and lung adenocarcinomas, via the MAPK and Akt pathways, which is also in agreement with the clinically significant cases where high Ca^{2+} has been observed in K-Ras4B dependent cancers.[20] According to Nussinov et al., CaM temporally forestalls Raf and MAPK and promotes PI3K/Akt activation, proliferative signaling and cell migration.[3] This information if studied in detail, could provide great insight in developing therapeutic strategies that regulate K-Ras4B-CaM interaction or CaM's signaling itself, based on the pathways being upregulated by CaM.

Predictive studies done on the interaction of K-Ras and CaM by Nussinov and co-workers has clearly provided some idea about the K-Ras4B-CaM binding. Their work on the predictive analysis of the interaction between CaM and K-Ras4B, done with the use of the hot-spot prediction tool called Hot Regions, indicates how the farnesylated highly variable region (HVR) of the K-Ras4B isoform alone, and not that of any other isoforms of Ras, is docked in the CaM's hydrophobic pocket. The role played by CaM is crucial in downregulation of the Raf/MEK/ERK pathway and PI3K/Akt pathways, which incidentally are often deregulated in cancer; Moreover, given that Ras is constitutively activated and that the Raf/MEK/ERK pathway and PI3K/Akt pathways are dysregulated in ductal adenocarcinomas, the chances of these processes being regulated by Ca2+/CaM could be considered high.[3]

CONCLUSION

The interaction of K-Ras and Ca2+/Calmodulin has the ability to affect downstream cell signaling in myriad ways temporally, that are overwhelming to study together. These interactions can efficiently control the fate of an individual cell as well as how it interacts with its microenvironment. Thus, the complications that can arise from induced K-Ras-CaM interactions like that might be triggered during tumor initiation or by particular drugs are alarming. Extremely meticulous structural studies and computational analyses should be conducted, complemented by specific cellular studies and backed by clinical investigations, to study K-Ras-CaM interactions and the signaling pathways triggered consequently, that will help to develop strategies of blocking the K-Ras-calmodulin interaction and could be of immense therapeutic value in the treatment of this dreaded category of cancers – the ductal adenocarcinomas.

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