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CHFR: A Silenced Suppressor Gene

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CHFR, an oncogene that is a current phenomenon in biomedical sciences, regulates the mitotic checkpoint during cell replication. Better known as a suppressor gene (the good guy), CHFR causes the delay of prophase at the mitotic checkpoint and a delay in cell replication, preventing the spread of tumorous disease. Oncogene, another term used to describe CHFR, describes normal genes that can be deactivated and cause an exponential growth in tumor cells due to a lack in mitotic checkpoint. CHFR is silenced (deactivated) by CpG methylation (consisting of nucleotide bases Guanine and Cytosine) using primarily methyltransferases DMNT1 and DMNT3b, which transfer a methyl group from a donor to an acceptor. Once the CHFR gene is silenced, the NF-kB pathway is activated and tumorigenesis proteins like IL-8 and HDAC, and the binding protein PARP-1, become increasingly abundant in the new tumor cells, creating malignancies. As stated above, the CHFR is quickly becoming the centerpiece of cancer research as its function proposes many questions. Researchers are designing multiple experiments that focus on what occurs in silenced CHFR gene cells, and ways to permanently activate the gene allowing it to resume its role at delaying prophase at the mitotic checkpoint. Past research has focused on the function of the CHFR gene and the components its deactivation can affect. By observing CHFR knockout mice, performing assays on lysed cells, immunoprecipitations, etc.; researchers have obtained a better understanding of the CHFR gene and the effects on malignant cell division.

Introduction

CHFR's main function occurs at the mitotic checkpoint, where it delays prophase due to mitotic stress (Ariga). CpG, a component containing the nucleotide bases Guanine and Cytosine, deactivates the gene via methylation (DMNT1 and DMNT3). Methylation causes binding proteins such as PARP-1 (poly(ADP ribose)polymerase 1) and tumorigenesis proteins like HDAC (Histone deacetylases) to become over expressed in tumor cells and deacetylate histones and other proteins, causing pathological disease to flourish. The CHFR gene consists of three domains: the fork-head N-Terminal FHA domain, a RING domain, and a C-terminal cysteine rich domain (CR), which is not fully elucidated. These domains are all deactivated when CpG silences the CHFR gene. The N-Terminal FHA domain regulates the mitotic checkpoint and phosphoprotein interaction, while RING domain contributes to the ubiquitin ligase activity of E3 and targets certain proteins for degradation (Ariga). Because science has focused on the RING domain and FHA domain, little research has been performed on the CR domain.

Once the CHFR gene is inactivated (silenced), tumor cells begin to replicate due to the lack of a mitotic checkpoint (an arrest at prophase), allowing defected chromosomes to replicate and pathogenic cells to divide. Recently, researchers have switched focus from study of various types of oncogenes to more experiments observing the function of tumorigenesis proteins and their designated pathways within pathogenic cells. Tumorigenesis is defined as the production or formation of tumors. By studying transcriptional regulation of CHFR (Idogawa), results about the HDAC and PARP-1 proteins have come to light. For example, both HDAC and PARP-1 are both considered tumorigenesis proteins and have shown an increase in numbers in silenced CHFR cells. PARP-1 is a CHFR binding protein, but has been

found to be numerous in cancer patients, making doctors and researchers classify it as a tumorigenesis protein.

In order to obtain a better understanding of both domains of the gene, recent experiments have shifted focus from the RING domain to the FHA domain of CHFR. Focusing on the FHA domain has presented more information on the pathways that are suppressed by an activated CHFR gene and the tumorigenesis proteins that become abundant in cancer cells. For example, HDAC (an enzyme protein) is found abundantly in CHFR silenced cells. By conducting experiments, using PCR, immunoprecipitations (Western Blots to test the concentration of proteins in cells), microassays, X-Ray Crystallography, etc., researchers are able to look at the CHFR silenced cells and better understand the way they function and experiment with inhibitors (pharmaceutical drugs) as a form of cancer therapy.

Recent Progress

Because of the fascinating function of the CHFR gene, scientists are curious to discover ways to permanently activate it, making the oncogene one of the top research topics in today's oncology field. The latest progress has shown that there are ways to inhibit the growth of cancer cells and deactivate the proteins abundant in tumor cells (i.e. HDAC and PARP-1). The FHA domain plays a major role in transcription and prohibits the signaling of the NF-kB pathway (nuclear factor-kB) (Ariga). This was discovered in an experiment that looked at the IL-8 (Interleukin-8 a pro inflammatory chemokine and a target of NF-kB), which is significantly down regulated by an activated CHFR gene (Kashima). By carrying out a cDNA microarray analysis, Kashima and his team were able to see effects of the CHFR gene regulation. By injecting a NF-kB reporter plasmid and deletion constructs of CHFR into CHFR gene expressing cells (HCT116 and HEK293) the team of researchers was able to look at both domains of the CHFR gene and their function when in the presence of the NF-kB pathway. It was concluded that the RING domain was not important in suppressing the NF-kB pathway, but the FHA domain was. They then carried out IL-8 promoter reporter assays to identify regulatory elements within the IL-8 protein necessary for its down regulation (Kashima). By cloning a base pair of IL-8 containing transcription factors and inserting it into upstream of a reporter gene, results were given explaining there are two nucleotides that contain the NF-kB pathway which are important for the down regulation of the IL-8 protein. The NF-kB pathway is an integral role in cancer cells; its activation allows the progression of cancer and more signals that allow for more biological activity of tumorigenesis proteins, such as IL-8. By silencing the CHFR gene and the FHA domain, transcriptional errors are occurring, creating defected sequences that are being replicated as tumor cells. However, Kashima's team was able to draw results concluding that by inhibiting the NF-kB pathway, using the FHA domain, IL-8 proteins are down regulated and the CHFR gene is able to do its job and delay prophase, prevents the spread of tumor cells.

HDAC (histone deacetylase), an enzyme that deacetylases histones and other proteins, causes: pathological pathways, proteins, and cells to divide, increasing the spread of cancer. Paola Infante and her team researched the affect of the tumorigenesis protein and its role in pathology. Infante found that HDAC proteins are misregulated in cancer, and used her research to look for protein inhibitors that would act as a form of cancer therapy to activate the CHFR gene and rid the body of cancer cells. Primarily Oncogenic fusion proteins recruit HDAC (Infante) and create malignancies within the body, making proteins like HDAC misregulated. The experiment looked at different protein inhibitors using microassays, PCR, ubiquitination, etc. The results included three protein inhibitors that are promising as forms of cancer therapy. By targeting the Hh pathway in brain tumor cells (Infante) they were able to look at the effect of inhibiting drugs. Although this was a base experiment, used to test the affect of inhibitory drugs on HDAC proteins, future experiments are able to use results from Infante's research and create experiments that reveal more detailed functions of the FHA domain and tumorigenesis proteins, and will propose new forms of cancer therapy.

PARP-1, poly (ADP-ribose) polymerase 1 is a CHFR binding protein (Idogawa). PARP-1 aids in DNA repair, cell cycle checkpoint control and apoptosis (Idogawa) but has also been found abundantly in human cancers. By using CHFR knockout mice, in vitro assays, and spectrometry, researchers were able to understand the relationship between CHFR and PARP-1. The purpose of this study was to identify the relationship between the PARP-1 protein and CHFR gene, which regulates the mitotic checkpoint and tumorigenesis. Idogawa used SK-BR-3 cells, HeLa cells, HSC-3 cells, HSC-44 cells, etc. that are all different types of cancer cells. These cells were transfected with siRNAs or other plasmids and performed immunoblots to look at the concentration of proteins in cells. Idogawa also used CHFR knockout mice to view the effects of growing tumors within these mice and the effects of certain concentrations of medicine in decreasing the diameter and or mass of the tumor (i.e. vincristine). The team of researchers then performed in vitro assays of immunoprecipations (Western blot of proteins that shows concentrations in certain cells) by immunoprecipitating the Myc-CHFR and PARP-1 with anti-Myc resins. Concentrations of proteins were then looked at and evaluated for the cancer cells used. Post research, Idogawa and team were able to confer that the

CHFR gene does interact with the PARP-1 protein and that an overexpression of CHFR genes creates a lack in PARP-1 proteins. This suggests that CHFR promotes polyubiquitination and degradation of PARP-1 (Idogawa). By degrading PARP-1 there is a stronger arrest in cell cycle prophase, displaying the results of an activated and working CHFR gene. However, silencing the CHFR gene could cause the overexpression of PARP-1, as seen in cancer patients.

Recent progress has focused much more on the FHA domain of the CHFR gene. By focusing on this domain, science has been able to look at the NF-kB pathway and down regulation of its proteins (IL-8) when CHFR is activated. Following this further, researchers have a deeper understanding about the FHA role in transcription, and without the domain, transcriptional error will occur. Furthermore, explanations are now available as to what occurs when the CHFR gene is silenced and how these tumorigenesis proteins are able to be treated (i.e. protein inhibitors via pharmaceutical drugs). On the other hand, science is still in the dark when it comes to the exact function of these tumorigenesis proteins and their role in tumor cells. But, there is still more research to be done, allowing for more knowledge in the role of CHFR and mitotic stress, as well as how to permanently activate the CHFR gene and hinder the spread of cancer cells.

Discussion

The results from the experiments above demonstrate the necessity of the CHFR gene to assume its role at the mitotic checkpoint and prevent the replication of tumor cells. By using knockout mice, microassays, western blots (immunoprecipitations), etc., as methods or research, experimenters were able to see how the NF-kB pathway functions as a result of silencing the CHFR gene and the increase in number of tumorigenesis proteins (i.e. PARP-1 and HDAC, IL-8) in tumor cells. The results of each experiment, although each tested different aspects of silenced CHFR cells, had the same conclusion: the CHFR gene is necessary for the arrest of prophase to prevent the spread of tumor cells. However, if the gene is silenced and malignant cells spread, there are ways to fight it and restore CHFR to its original function.

Pharmaceutical drugs act as a form of cancer therapy. These drugs serve as protein inhibitors in cancer patients that fight the tumorigenesis proteins, and pathways such as NF-kB, and reactivate the CHFR gene. By conducting research, such as the experiments discussed in this review, scientists are able to obtain a better understanding of the CHFR gene and its affect on malignancies. The research above leads to even more questions, such as: how can the CHFR gene remain activated indefinitely and what other oncogenes exist within the human body. Furthermore there are more tumorigenesis proteins in which research could focus on besides PARP-1 and HDAC; for example, the EXO-C7 gene.

The results given in the above experiments and from other smaller projects pave a pathway for further research of oncogenes, especially CHFR and the NF-kB pathway and it's tumorigenesis proteins. By using these results as a basis for future projects, we can create more detailed experiments that show specific functions of tumorigenesis proteins and pathways in cancerous cells. With future knowledge, science will be able to find specialized medicine to fight diseases such as cancer, as well as chronic diseases such as Cerebral Palsy and diabetes.

There is hope that in the future, research will uncover more information about ways to indefinitely activate the CHFR gene. But for now, we know it is necessary for the CHFR gene to remain activated, primarily for its function at the mitotic checkpoint, which delays prophase and prevents the reproduction of tumor cells and spread of cancer. We also know that there are types of cancer therapies using pharmaceutical drugs that act as protein inhibitors. The experiments in this review serve as a base for future experiments, while at the same time helping current medicine. With more research come more answers; but for now, doctors, researchers and other medical professionals will have to remain content with the current results.

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