



MASSACHUSETTS
GENERAL HOSPITAL
CANCER CENTER



TargetCancer

The TargetCancer Cholangiocarcinoma Cell Line Bank at Massachusetts General Hospital

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Relatively few studies in the literature have focused on the biology or genetics of cholangiocarcinoma (CHC) in comparison to better known cancers such as breast, colon, lung, and prostate. Consequently, there is a deficit in understanding the processes leading to CHC development as well as a paucity of experimental systems with which to define these processes. Such an understanding is critical to developing more effective and specific therapies.

Why a cholangiocarcinoma cell line bank?

To address which gene mutations are drivers of CHC and to define the genetics of drug sensitivity in this cancer type, it is important to have a series of cell lines derived from CHCs that can be grown and studied in the laboratory. Cell lines from multiple patients are necessary for these studies to fully account for the genetic variability that is seen between individual CHCs. While there are dozens or hundreds of cell lines derived from lung cancer, melanoma, and breast cancer, there are only a very small number of CHC cell lines available. To illustrate the scale of the problem, the Center for Molecular Therapeutics at Massachusetts General has a collection of more than 1000 cell lines obtained from the main cell line repository in the United States (the ATTC), including more than 200 lung cancer lines, whereas only 2 are CHC lines.

To expand this collection, we have sought to obtain additional lines by contacting investigators around the world. With some difficulty, we have now been able to obtain approximately 15 CHC lines in this manner. Despite these efforts, there are several limitations to our current collection. Firstly, since CHC can be divided into subtypes with very different biology (a type arising within the liver and a second type in the bile duct outside the liver), our collection of lines only has a modest representation of each type of the disease. Secondly, all but two of the cell lines we have originated from patients in Asia. Since it is clear that the environmental risk factors for CHC are very different in North America and different Asian countries, it is important to have a better representation of cell lines derived in North America. Additionally, the cell lines that are generally available have been maintained in the laboratory for a very long time (some of the current lines date from the 1980's), and are likely to have developed some changes from their originating tumor during this time. Finally, in most cases, there are very limited clinical information available associated with the initial cancer from which the cell line was derived, therefore, the cell lines in general use are quite poorly defined as experimental systems upon which to base conclusions about patient's tumors. In fact, in many cases, it is not possible to say with certainty whether an individual cell line is derived from CHC or an entirely other cancer type.

What can be learned from these cell lines?

Study of new 'driver mutations'. A major area of advancement in other cancer types has been the discovery of new gene mutations through the use of recently developed DNA sequencing technologies. These technologies have greatly reduced both the cost and time required to identify the full spectrum of gene mutations in a given cancer cell. Many groups around the world are currently applying these sequencing approaches, and in the near future, we will have a detailed genetic

blueprint for each type of cancer, including CHC. However, a critical question going forward is to define which of these mutated genes play central roles in the continued, aggressive growth of a cancer cell, and which are either 'bystanders' that are picked up as the cancer develops but don't contribute to its growth, or are involved in the early stages of the cancer, but are dispensable once the cancer is fully formed. The genes that are critical for the sustained growth of cancers can be thought of as 'drivers' of cancer. Such drivers would be ideal targets for new drugs since they are both uniquely mutated in the cancer cells and essential for its growth and survival.

Personalized medicine. In addition to the issue of which genes may be cancer drivers, another key question is how the different combinations of mutations in a given tumor influences the response to other drugs (including both those that directly target the mutation and those that act by other mechanisms). This question is important since there is variation between individuals with the same cancer type— for example some CHCs have mutations in a gene called KRAS, while other have mutations in the PIK3CA gene. These and other mutations are likely to influence how a cancer responds to a given drug or combination of drugs. With this information in hand, it becomes possible to establish personalized therapies such that the genetics of a patient's tumor determines which drugs to employ.

How will this work?

The TargetCancer Cholangiocarcinoma Cell Line Bank will develop new CHC cell lines from patients treated at Massachusetts General Hospital, and other participating area hospitals, through a partnership between Dr. Nabeel Bardeesy (research), Dr. Cristina Ferrone (surgery), and other clinicians and pathologists. To date, we have optimized approaches to generating new CHC cell lines using both primary resected tumors (those derived from surgical specimens), and from ascites (cells that have disseminated into the abdomen). This project creates a unique repository of CHC lines representing the North American patient population and provides a valued research model of CHC. We will employ them for studies aimed at investigating new CHC gene mutations and drug combinations (as mentioned in the "driver mutations" and "personalized medicine" sections above). Importantly, once the lines are generated and characterized we will establish a bank to facilitate sharing them broadly with the CHC research community.

Cell lines will be assessed for mutations in the set of genes most commonly mutated in cancers, and we will confirm that the primary tumor and cell lines share the same mutations. For some of the studies with ascites, we will use flow cytometry to isolate subpopulations of tumor cells based on cell surface makers. This latter experiment will allow us to test the notion that there exist more primitive cell populations within the tumor that have stem cell-like features and may have different drug sensitivities. The tumorigenicity of the cell lines will be confirmed by growth in soft agar or injection into immunodeficient mice. In addition, a subset of the primary tumors will be injected directly in mice to establish xenografts models.