Chapter

Discovery of BRCA Mutations: Historical Perspective of Its Scientific, Clinical and Social Impact

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Abstract

In the human genome, BRCA1 and BRCA2 (for **BR**east **CA**ncer 1 and 2) genes encode for proteins involved in several functions that are crucial for the maintenance of genome stability and integrity. They participate in DNA damage response and repair pathways and, therefore, act as tumor suppressor genes. Mutations in these genes, which are located in chromosomes 17q21 and 13q13 respectively, are responsible for a great fraction of inherited breast and ovarian cancers, as well as other pathologies, such as Fanconi Anemia. Approximately 30 years ago, a report from a group of the School of Public Health at the University of California about a hypothetical gene that led to predisposition to early-onset breast cancer in certain families changed the history of breast cancer research, diagnosis, and prevention. Nowadays, the accessibility of genetic testing and the availability of different approaches as wide coverage screenings, prophylactic mastectomies, and risk-lowering drugs benefits BRCA1 and BRCA2 mutation carriers enormously. This chapter summarizes the unique trajectory of BRCA research and its scientific and social implications.

Keywords: history, BRCA1, BRCA2, breast cancer, ovarian cancer

1. Introduction

Breast cancer is the world's most prevalent cancer type. In 2020, 2.3 million women were diagnosed with breast cancer and 685.000 deaths were reported globally, ranking as the most common cause of cancer death among women [1]. From the 1930s to the 1970s breast cancer mortality rate remained quite constant, but since 1980, thanks to the implementation of early detection procedures, there has been notable progress in survival rates. Nowadays, some risk factors that lead to breast cancer are well identified and, in general, the population is better aware about them, as the information became more available in the era of digital communication. However, this progress has not been even among different regions in the world. Data suggest that the low income countries have diminished survival rates due to less access to information and early testing [2].

The high rates of breast cancer incidence and mortality in women of certain families led to a long search for the causes of inherent susceptibility to develop this illness. Around 1970, a global race to discover those factors started and reached the first milestone in 1994 that was the Science publication reporting BRCA1 sequencing, which was soon followed by the discovery and sequencing of a second gene, BRCA2. This chase indelibly marked the cancer and genetic research fields, but also labeled the initiation of the ongoing discussion about the role of private companies on gene discovery and patenting. Since then, medical progress based on gene and mutation discoveries have involved not only scientific, but also major ethical and commercial debates. Now, almost 30 years later, those issues are still controversial. In addition, and in spite of the huge advance of basic knowledge about those genes, several fundamental questions remain unanswered, such as why, in people carrying a mutated BRCA gene copy, hormone-sensitive tissues are the most prone to neoplastic transformation. In this chapter, we will focus on these topics, which are still under debate.

2. Historical aspects about the discovery and diagnostic usage of BRCA1 and BRCA2 mutations

In December of 1990, Mary Claire King's group published a research article reporting the analysis they performed in 23 extended families with a very high incidence of early-onset breast cancer. Analyzed cases were chosen meticulously according to their pathology records. Using different polymorphic markers and four statistical approaches, they associated those tumors with alterations in chromosome 17q21 [3]. Is important to underscore that only the position was then reported, and without the complete human genome sequenced, the gene that caused early-onset breast cancer was merely hypothetical. Then, the "race" to specifically establish the mutated gene responsible for hereditary breast cancer syndrome began.

A year later, Gilbert M. Lenoir and his colleagues confirmed King's team finding, but also associated that chromosomal location to a proportion of hereditary ovarian cancers [4] and Mary Claire King named this still hypothetical gene BReast CAncer 1 (BRCA1). In 1992, King's group reduced the previously located area to a 8-cM region that was very likely to include this gene. They pointed out that it would be a mistake to oversimplify and consider that mutated BRCA1 was involved in breast cancer initiation in the general population, since the analyzed cases corresponded specifically not only to families with high breast cancer incidence, but also to patients with early-onset disease. In addition, they indicated that the data that linked the breast cancer syndrome to chromosome 17q was heterogenous, suggesting that it might be another gene involved in the development of familial breast cancer, and/or associated to a high frequency of sporadic cases in older-onset families. Importantly, they also reported that it was not possible to distinguish sporadic to familial breast cancer by clinical criteria. Therefore, the involvement of BRCA1 in the inherited risk of mammary tumor development was solely defined by the identification of mutations in breast cancer patients of susceptible families [5].

It was not until October 1994, that the predicted amino acid sequences of BRCA1 and some probable predisposing mutations were published by Mark Skolnick *et al* in *Science* [6]. Up to that time, King's laboratory as well as other groups were close but not able to sequence the whole gene, yet they were later responsible for identifying several different mutations in affected families. Importantly, a month earlier that same year (September 1994) the BRCA2 gene was identified in chromosome 13q

through a similar strategy to the previously used, but analyzing 15 families with multiple cases of early-onset breast cancer not linked to BRCA1. Interestingly, men bearing BRCA2 mutations showed higher breast cancer risk, but women had less chances to develop ovarian tumors than BRCA1 mutation carriers [7]. The following year, *Nature* published the BRCA2 predicted amino acid sequence as well as this gene mutations based on the Human Genome Project as well as data provided by the Sanger Center and Washington University [8].

Localization and sequencing of the BRCA genes did not provide any clue about the possible biological roles of the proteins encoded by them. They neither showed homologies to any other protein characterized up to that date, nor distinguishable functional domains. Therefore, it was not rapidily elucidated which were BRCA1 and BRCA2 biological roles and whether they overlapped totally or partially. Although the proteins are not similar in their primary sequences, they share several particular features. For example, both are surprisingly large and highly charged, and the corresponding genes have many exons, which suffer alternative splicing. Interestingly, exon 11, where the interactive regions with RAD51 are located, is particularly large and encodes about half a protein in both cases. Curiously, if not for the classic genetic approach to detect them, neither BRCA nor BRCA2 would have been selected as tumor suppressor candidates for to the information provided by their sequences.

The data that arose soon after their discovery were mostly related to the impact of different BRCA1 and BRCA2 mutations on cancer risk. It was determined that some subpopulations with a high tendency to develop breast cancer carried alternative genetic variants in their germline and that the prevalent mutations had been established as a consequence of a founder effect. This happens when a small group of people remains separated from the original population and, after several generations of interbreeding, rare mutations present in the first generation become more frequent. For example, Ashkenazi Jew families, whose ancestors lived in Central and Eastern Europe, are particularly affected by three well-known founder mutations; BRCA1-185delAG, 5382insC, and BRCA2-6174delT, with an overall rate of 2.6% (1/40), in contrast to the 0.2% (1/500) of these three BRCA1/2 mutation carriers in the general population [9]. Other founder mutations have been determined in various European populations, such as some Norwegian, Dutch, and Icelandic families [9, 10]. The possibility of having information about large numbers of people with the same mutation opened the door to analyze the penetrance of such variants, together with the importance of risk-modifying factors that could affect the outcome of the disease [9].

By the age of 70, women who carry a BRCA1 or a BRCA2 clinically relevant mutation, have a 50–65% or 50–55% chance, respectively, of developing breast cancer, while that probability goes down to 7% for women without any of those mutations. In the case of ovarian cancer, the risk is between 35 and 70% for women with a BRCA1 gene mutation, it is lower (10–30%) for those who bear a BRCA2 mutation, but less than 2% for women who do not have any of those variants [11]. For men, only BRCA2 mutation carriers have a significantly higher risk of developing breast or prostate cancer. However, all people who possess one BRCA1 or BRCA2 mutation have an increased risk of developing pancreatic cancer and Fanconi anemia. An increased susceptibility to melanoma has been observed only in the case of BRCA2 inherited gene mutations [12]. It is important to remember that BRCA mutations can also occur sporadically.

BRCA1/2 mutation carriers have between 40 to 80% more chances of getting a second primary contralateral breast tumor. However, it is remarkable that not all

women carrying a BRCA1 or BRCA2 mutation get breast cancer. Therefore, there are other risk factors involved in the occurrence of this illness, even for women who inherit the harmful mutations. Interestingly, BRCA1 and BRCA2 female carriers tend to develop different breast cancer subtypes. The first are more likely to have triple-negative tumors (*i.e.* Estrogen receptor-negative, Progesterone receptor-negative, and HER2-negative), which do not have specific clinically successful treatments, while the latter show a higher probability to develop estrogen receptor-positive (*i.e.* luminal) breast cancer, that usually receive endocrine therapy [11].

Even though most breast cancers do not involve a specific hereditary component, in particular cases it is recommended to take a genetic test in order to find out whether BRCA1 or BRCA2 is mutated in the germ line [11]. This analysis (patented on 1997 in the US) and owned by Myriad Genetics until 2013, is commonly recommended only under certain conditions, such as high incidence of breast or ovarian cancer in the family, belonging to certain ancestries (*i.e.* Ashkenazi Jew), etc. Nonetheless, until 2013, a critical aspect for which the test was not massively advised was the price, which was over US$3000. Undeniably, a value not accessible to everyone. In addition, there were many other elements that the clinicians took into account before prescribing the BRCA1/2 genetic test, as the psychological impact of the result on the patient and their family as well as her/his predisposition to undergo prophylactic measures.

Almost from the beginning, it was clear that gene testing was not the magic answer to all clinical questions. In 1997, a review article clearly showed the problems associated with gene testing, which can be summarized into two main points intrinsically related: one related to technical and biological issues and the other associated to understanding and usefulness. Specifically, for the detection of BRCA 1/2 mutations, technology available in the late 90s was still slow, expensive and not too sensitive. In addition, there were variants still unknown and/or with no existing information about its biological association with cancer [13]. Genetic testing carries complex issues that even today are misunderstood by the general public and health professionals, so it is necessary to provide the required knowledge to both. Otherwise, gene testing may result useless, cause a waste of resources and be dangerously utilized.

The result of the gene test can be positive, negative, or indicate a variant of uncertain significance (VUS, when the harmfulness of the detected mutation is unknown). If a known variant is found, their relatives can be tested to determine whether they carry the same mutation, which is less expensive than sequencing the whole gene for each of them. In many cases, health insurance covers BRCA testing if the person meets the established criteria. With an accurate diagnosis and treatment at an early stage, women who have a BRCA1/2 inherited gene mutation present a similar survival rate than those who develop breast cancer without an inherent genetic component [10, 11]. In recent years, the recommendation of testing based only on familial high cancer incidence has been questioned. According to a report published in 2015, half the breast cancer patients with BRCA1/2 mutations did not meet family history criteria for testing, so they learned they were carriers after cancer had already developed. This was a breaking point in cancer prevention and, based on those and other similar results, some researchers and clinicians encourage population screenings that enable a much more complete and cost-effective identification of carriers [14].

In a study carried in 1999, among 200 women with breast and/or ovarian cancer, who were offered testing for BRCA1 and BRCA2 free of charge, a high proportion of them had overestimated their risk of having a mutation, and some of them faced difficulties with their health insurance if the outcome of their analysis resulted positive [15]. These facts reflect how the lack of general knowledge about genetics,

and particularly, cancer genetics may lead to unnecessary psychological stress, and the economical and social pressure suffered by who is (or suspects to be) a BRCA1/2 mutation carrier. A boom of testing occurred right after May 2013, when Angelina Jolie shared her breast cancer family history in the *New York Times* [16]. In an opinion article, the well-known actress and director, announced that upon learning that she carried a BRCA1 mutation, she decided to undergo double mastectomy to reduce her risk of dying from cancer. Later, she also had her ovaries and fallopian tubes removed. In that commentary, she encouraged women to take a genetic test if they believed they were highly susceptible to develop breast and/or ovarian cancer and, in that way, to take active action to prevent the onset and progression of these diseases. According to doctors and medical centers, this event significantly increased BRCA testing and public interest in this subject, a phenomenon baptized by the media as the "Angelina Jolie effect" [17].

There are different options for BRCA1/2 mutation carriers to decrease cancer risk, such as taking early detection tests and/or undergoing surgeries, like prophylactic mastectomy or oophorectomy (or both). When the first women diagnosed with those genetic variants had to make a decision, the data on the long term outcome of these procedures were very limited. Nevertheless, many social and clinical studies about their efficacy have taken place since then. In 1997, a special report showed that prophylactic mastectomy led to a higher increase in life expectancy than prophylactic oophorectomy, but there were much better benefits when both procedures were done [18, 19]. On the other hand, the advantages of those procedures tended to decrease with increasing age, being almost not significant at all when they were performed in women over 60 years old. That is why BRCA1/2 mutation testing is recommended for women under that age [19].

Some women may avoid BRCA testing for fear of the adverse effects of prevention treatments if they resulted positive. Breast and/or ovaries removal may affect them physically and emotionally as it may lead to fertility problems and issues with their body image. Oophorectomy causes early menopause, which can induce weight gain, an increased risk of cardiovascular diseases, osteoporosis and sexual discomfort. In addition, tamoxifen and raloxifene tratments have rare but severe side effects, such as uterine cancer, blood clots, and stroke [17]. Therefore, precise and early advice about pros and cons of these preventive approaches is required so candidates for these procedures can choose what is best for themselves [19].

Presently, prophylactic mastectomy can reduce the risk of breast cancer by over 90%, but for those who do not want to go through surgery, it is advisable to take a yearly screening with breast magnetic resonance imaging (MRI) (or mammography, which is less recommended because a harmful BRCA variant might be particularly sensitive to the DNA-damaging effects of radiation), as well as biannual pelvic ultrasonography and cancer antigen 125 (CA-125) testing. Noteworthy this protein has been identified in women with advanced ovarian cancer, but it has not been found in early stages of this illness, therefore screening based on its detection has not improved survival [10].

BRCA1/2 deficient cells may be sensible to classic chemotherapeutic drugs that arrest replication and DNA cross-linking agents, like those based on platinum components. Therefore, these drugs, may be recommended to mutation carriers [20]. Since normal BRCA1 protein participates in DNA repair mechanisms by homologous recombination, in the past few years, new drugs (like olaparib and talazoparib), which inhibit PARP1 enzyme and therefore block DNA reparation pathway by base excision repair, have been developed. These drugs leave a gap in the single strand

DNA that arrest the replication fork and convert the single strand break into a double strand break. This leads to cell death in cells whose repair mechanisms by homologous recombination are damaged because of BRCA1 function failure. Currently, there are four PARP inhibitors approved for clinical use, although it is essential to continue investigating possible new synthetic therapeutic and lethal targets, because PARP inhibitors have toxic effects on normal cells and some tumor cells may be resistant to these treatments [20, 21].

Shortly after BRCA1 sequencing was completed, Myriad Genetics, a biotechnology and molecular diagnostic company founded in 1991 in the US, requested patents over BRCA1 gene and over BRCA2 later on. They were granted in 1997 for the US, in 2000 for Canada and in 2001 for Europe, obtaining seven patents in total. In the country or countries where an invention has been patented, their owners control its making, using, and selling for a specific period, which, in the case of BRCAs, corresponded to twenty years. Laws of nature, physical phenomena and abstract ideas are not patentable. However, the BRCA applications were granted because the US Patent and Trademark Office (USPTO) argued that isolated human gene sequences were patentable because human labor was needed to extract and purify them. Noteworthy, a similar stance was taken by the European Union.

BRCA1 patent provided Myriad Genetics the rights over the diagnostic or therapeutic use of this gene, whatever technique was utilized for carry on the assays, as well as all mutations found in familial breast and ovarian cancers, and their usage for determining cancer predisposition. Similarly, BRCA2 most frequent allelic variants, identified mutations associated to disease and methods for determining nucleotide sequence variations were protected [22]. Therefore, Myriad had a wide span of patent rights that provided them the monopoly for BRCA testing [23]. The company required all laboratories to send the DNA samples to Myriad headquarters in Salt Lake City, Utah for testing. That involved an initial cost of 1600 US$ and they could decide what research might be carried out on those genes, by whom and how much any resulting therapy or diagnostic test would cost [24]. This arrangement, which also included complete control over any further research on the diagnosis of certain breast tumors, was unprecedented in the field of genetic testing [22]. Furthermore, Myriad promoted the BRCA test to physicians and to the general US population on TV and print media, causing unnecessary anxiety about breast cancer risk [25]. In September 2007, the company released a questionable direct-to-consumer (DTC) marketing campaign offering genetic testing for 3100 US$ without requiring personal medical advice for its solicitation. Noteworthy, the prices were increased while new technology actually made testing less expensive [26]. However, many laboratories performed diagnostic BRCA1/2 tests without observing patenting rights, putting themselves at risk of being sued.

With the aim of canceling Myriad patents, many plaintiffs coaligned across different countries. Europe was the first to invalidate the BRCA patents with different arguments, including the Art. 52 (4) of the European Patent Convention: “Methods for treatment of the human or animal body by surgery or therapy and diagnostic methods practiced on the human or animal body shall not be regarded as inventions ...” and focusing on three points: Firstly, the lack of priority and absence of novelty, because gene sequences were already available in public databases when the third patent, which covered a specific set of mutations related to familial breast and ovarian cancer and their use in methods for determining predisposition for breast and/or ovarian cancer, was filed; secondly, the lack of inventiveness, for the same reasons indicated above, and thirdly because therapeutic uses of mutation sequences, in particular gene

therapy methods, were not sufficiently described for implementing them effectively. Also, by 2002, the Institut Curie demonstrated that the methods used by Myriad Genetics to detect mutations, failed to identify about 10-20% of all expected mutations [22, 23]. In addition, Dr. Mary Clair King's article in JAMA proving that Myriad's tests missed a significant number of mutations, discredit this company and its rights on BRCA1/2 in the UK and Canada. In the US, the news were mainly covered by Utah from the perspective of Myriad, who rebutted the claim [27].

In 2010, a US District Court stated that all BRCA patent claims were invalid and that isolated DNA is 'not patentable subject matter'. Then, in 2011, the US Court of Appeals for the Federal Circuit overturned this ruling (2 to 1 decision); but in 2013, the Supreme Court decided to re-examine the lower courts' decisions and to analyze the claims on gene patenting [28]. Finally, the Supreme Court agreed with the plaintiffs that isolated gene sequences were not patentable because they were not "markedly different" from gene sequences already existing in nature [29].

With the expiration and overturning of BRCA patents, there were no more limitations on the offering of commercial testing. Also, these legal changes occurred approximately at the same time as the widespread adoption of massively parallel sequencing (MPS) technology, which allowed less expensive testing panels featured in turn-around times (TAT) [30].

At this point, we would like to go back to the woman behind this ongoing story, Mary-Claire King, whose contributions for society exceed the discovery of the BRCA genes. Born in 1946 in the US, she attended college and got a degree in mathematics inspired by two of her high-school teachers. Then, she went to the University of Berkeley for graduate school, where she took a genetics course and decided to follow that path. It was the 60s and a lot of social and political situations were taking place all over the world, and the States were not the exception. King was involved in social justice causes like the civil rights movement, and subsequently the anti-war movement. For her Ph.D. Thesis, mentored by Allan Wilson, one of the firsts to approach evolution from a molecular perspective, she demonstrated that humans and chimpanzees share 99% of their coding sequences [31], which caused an evolution revolution at that time.

In the early 1970s, Dr. King moved to South America with her husband in an exchange program between the University of California and the University of Chile. She taught genetics, statistics, and evolution until 1973, when a military coup overthrew President Allende's government. They could not stay, so the couple decided to return to the US. Back then, postdoc positions did not quite exist in academia, but as she said in a conversation with Ushma S. Neill, "*there were many jobs available in cancer research, because President Nixon had just launched the war on cancer. One of those jobs was at UCSF with a lovely pediatric oncologist named Nicholas Petrakis who had become interested in breast cancer*" [32]. Petrakis soon became her post-doc mentor and she started studying an inherited genetic component in breast cancer.

She obtained that position thanks to affirmative action, a policy aimed to balance gender inequality, a fact she noted in more than one interview. Her involvement in social justice was not only about her feminist position, but also her scientific collaboration in the investigation of human rights abuse. In the early 1980s, Dr. Cavalli-Sforza from Stanford, started teaching Dr. King, molecular genetics. And by that time, the Committee on Scientific Freedom and Responsibility of the American Association of Advancement of Science (AAAS), contacted him as a consultant for a genetic issue. In 1977, the Grandmothers of Plaza de Mayo (the "Abuelas", as they are commonly known in Argentina) had organized themselves in Buenos Aires during

the last civil-military dictatorship, to demand the return of their grandchildren, who were born during the captivity of their missing ("desaparecidas") daughters. The "Abuelas" obtained good anecdotal evidence from some survivors and other witnesses about how those babies were given to different families. Then, they correctly proposed that it should be possible to establish the biological familial link by DNA comparison of the grandparents with their putative grandchildren, even if the parents were missing and presumed dead. To reach that goal, Dr. King and her colleagues created a genetic test that provided an "index of grandpaternity" ("índice de abuelitud"). Dr. King (who was fluent in Spanish) traveled to Buenos Aires to put the grandpaternity test into practice in June 1984, and that is how it began a 30-year collaboration with the organization of "Abuelas de Plaza de Mayo" [32–34]. Since then, and thank to that test, 130 grandchildren have been found by their biological families. Presently, Dr. King keeps on working at the University of Washington, trying to solve hereditary cases of breast cancer that cannot be explained by BRCA1 or BRCA2, but rather cryptic mutations that remain elusive [35].

2.1 Understanding the biological role of the Breast Cancer genes

The early reported BRCA1 and BRCA2 mutations coded for truncated proteins, and the loss of the wild-type allele (loss of heterozygosity, LOH) was detected in tumor samples from affected families [36]. The scientific community agreed on these genes being tumor suppressors, although the nucleotide sequences revealed nothing about their protein function. Starting in 1996, different groups around the world dedicated their efforts to decode the cellular localization and biological role of the BRCA proteins, because the first step to figure out how their deregulation could lead to disease was to understand their activities in physiological conditions.

Mouse models revealed that BRCA1 and BRCA2 were required for embryonic cellular proliferation, as different homozygous mutations resulted in embryonic lethality. The KO embryos showed no increased apoptosis, but cell proliferation was impaired and strong upregulation of the cell cycle regulator p21 was observed. In contrast, mouse with heterozygous mutations were phenotypically normal and fertile up to almost one year of age, although it was not ruled out the possibility of tumors may develop at more advanced age. The similarities displayed by embryos with homozygous null alleles for either BRCA1 or BRCA2 led to think that both genes acted in the same pathway during embryogenesis [37, 38].

BRCA1 and BRCA2 showed an incredibly similar pattern of expression through cell cycle when studied in normal and tumor-derived breast epithelial cells [39]. In synchronized cultured cells, gene expression reached a maximum in late-G1 and S-phase, suggesting a role for both genes in cell proliferation and cell cycle checkpoints. Soon, the first clue that BRCA1 and BRCA2 were actually involved in the DNA repair and homologous recombination came from the observation of co-localization and co-immunoprecipitation of BRCA1 with human Rad51 (hsRad51) in cultured cells [40]. However, it was then determined that it is BRCA2 the one that directly binds to hsRad51, while the interaction with BRCA1 would be indirect, requiring the participation of at least another protein [41]. hsRad51 is the human homolog of *E. coli* RecA and Rad51 in *Saccharomyces cerevisiae*, which are involved in DNA recombination and damage repair. In mouse and mammalian cells, homozygous knock-out of Rad51 displayed a very similar phenotype to BRCA1−/− and BRCA2 −/− mice, indicating the involvement of that protein in cell viability [42].

It has been shown that BRCA1 and BRCA2 colocalize in nuclear foci of somatic cells as a biochemical complex. In addition, these proteins also coincides with hRAD51 in DNA replication sites after exposure to damaging agents [43]. Furthermore, mouse cells with truncated *Brca2* gene have shown not only G1/S and G2 phase arrest, but also aberrant chromosomal number and structure [44]. Interestingly, cell cycle checkpoints and apoptosis mechanisms displayed no alterations [44]. These reports reinforced the idea that BRCA1 and BRCA2 share some common roles, acting coordinately to preserve chromosome stability. However, there must be functional differences between them that would account for the observed variation in cancer risk for BRCA1 and BRCA2 mutation carriers.

In 1996 and 1997 it was reported that both BRCA1 and BRCA2 contained domains that, when associated with the DNA-binding domain GAL4, induced transcriptional activation in yeast [45, 46]. Moreover, it has also been demonstrated that full-length BRCA1 was a component of the RNA Pol II holoenzyme [47]. Therefore, it has been proposed that the BRCAs may participate in transcription regulation. However, it has not been determined yet whether this activity would be independent from the DNA repair function. In addition, it also remains to be solved why mutation in genes coding proteins involved in very basic activities, such as DNA repair, required in every single cell type, induce high cancer risk only in very specific tissues. To date, there is still no right answer to this question. There are different theories about tissue-specific carcinogenesis caused mostly by BRCA1, but also BRCA2, in mutation carriers. The analysis and implications of this open question exceed the purpose of this chapter, but we briefly report here the leading hypothesis on this subject.

Based on certain BRCA deletion mutations found in cancer patients, it has been proposed that tissue specific carcinogenicity would be due to the loss of "breast-cancer cluster" regions (BCCRs) and "ovarian cancer cluster" regions (OCCRs) that are present in both *BRCA1* and *BRCA2* genes. Nevertheless, this proposition should be taken cautiously, because deletions in the proposed sequences would not only eliminate downstream exons coding for protein regions, but also might repress protein expression through nonsense-mediated mRNA decay. Therefore, those mutations, specifically those corresponding to the first few hundred nucleotides of the *BRCA1* or *BRCA2* coding sequences, would probably result into functionally "null" alleles, which should not cause tissue-specific effects [48]. Alternatively, it has been postulated that the higher cancer susceptibility of Estrogen-dependent organs to BRCA mutations would be due to the genotoxic effect that this hormone may exert on cells. Then, it has been suggested that hormone-responsive tissues might be particularly sensible to the failure of DNA damage reparation exerted by BRCA proteins [49]. On the other hand, another theory is based on the accumulation of R-loops, DNA–RNA hybrids, necessary for the differentiation of normal mammary luminal epithelial cells. In brief, BRCAs would be required for recruitment of some molecules involved in transcription (*i.e.* BRCA2 is necessary for PAF1 activity) and, in the absence of BRCA proteins chromatin disassembly may decrease. Then, transcription elongation would be obstructed causing accumulation of RNAPII in promoter-proximal pausing (PPP) sites generating R-loops which would lead to DNA breaks and consequently to genomic instability [50]. Growing evidence indicates the connection between R-loops and estrogen activity. These reports point out that genomic instability, increased in the scenario of BRCA1 or BRCA2 mutations, may be especially enhanced in estrogen-responsive tissues such as the breast [51]. This establishes another possible explanation for the high risk of BRCA mutation carriers to develop tumors in those organs.

3. Conclusions and final remarks

As reported at the beginning of this chapter, breast cancer is a relevant issue due to its high incidence worldwide. Here, we summarized the very first steps that resulted in the discovery of BRCA1 and BRCA2 using basic genetic and statistics tools by many groups simultaneously. Then, the prognosis of having a mutation in these genes is explained and the strategies and treatments for cancer prevention in mutant carriers are indicated. Additionally, we report the legal history around the controversial patenting of these genes as well as a brief report about Mary Claire King a key scientist in BRCA discovery and in the recent history of our country. In the second part of this chapter, we review some BRCA1 and BRCA2 biological functions, particularly those relevant to the not completely answered question of why mutations in these genes cause high risk of developing tumors particularly in hormone-responsive tissues.

In summary, it can be said that the story behind the finding of BRCA1 and BRCA2 as well as its further scientific and clinical developments have not been linear at all. It have involved multiple actors as classical and molecular geneticists, clinicians, lawyers, entrepreneurs, ethical experts, pharmaceutical companies, and the judiciary systems of diverse countries. Undoubtly, is one of the best examples of how a scientific discovery may change human society for ever.

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