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Cancer genetics—one family at a time

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Abstract

This article is a distillation and extension of some of themes I presented as a lecture to the Canadian Society of Clinical Investigation meeting in Banff in 2019. I emphasize the important role that investigation of a few key families seen in our clinic (sometimes as few as one) can play in cancer genetic discovery. I present the work both thematically and chronologically, demonstrating how clinical observation, combined with laboratory work and extensive collaboration can lead to novel insights. I will focus on families possessing germline pathogenic variants in *PALB2*, *DICER1*, *SMARCA4*, *FGFR1* and *RAD51D* as examples of how the process has been carried out in our group.

For the last 30 years, inherited susceptibility to breast and ovarian cancer has been one of the most intensive areas of cancer genetics research. The high points were the discovery of *BRCA1* and *BRCA2* as the two major breast and ovarian cancer susceptibility genes, which were reported in 1994 and 1995, respectively [1,2]. Since then, thousands of papers have been published on these two genes, with surprising discoveries being published with astonishing regularity. It was, however, noted in the original linkage studies [3,4] that the development of cancer in some families with many cases of breast and/or ovarian cancer could not be attributed to *BRCA1* or *BRCA2*. The hunt was on for *BRCA3*. After 10 years of largely fruitless labour, it became obvious that a *BRCA3* gene with properties very similar to *BRCA1* and *BRCA2* does not exist [5]; however, that same year, a gene

was discovered—the partner and localizer of *BRCA2* gene (*PALB2*) [6]—which is undoubtedly the most significant breast cancer susceptibility gene discovered since 1995. Shortly afterwards, in 2007, three groups discovered pathogenic *PALB2* variants in families with breast and related cancers [7-9]. We went on to discover a French Canadian founder pathogenic variant, p.Q775Ter [10], and also reported the first known pathogenic missense variant in *PALB2*, p.L35P [11]. As part of the activities of the *PALB2* Interest Group (led by my colleague, Marc Tischkowitz), robust, clinically-useful estimates of breast cancer penetrance were published in 2014 [12], and are currently being updated. *PALB2* also turns out to be an important pancreas susceptibility gene [13,14] and is more similar to *BRCA2* than to *BRCA1* in several ways.

FIGURE 1. A family with multiple cases of early-onset breast cancer.

Figure 1. This was the first family in which we identified a *PALB2* pathogenic variant. The *PALB2* pathogenic variant was not found in tumor samples from the proband's mother (II.2), indicating that the causative pathogenic variant in *PALB2* was inherited from the paternal side. Abbreviations: MM, malignant melanoma; PSU, primary site unknown; *, unconfirmed cancer diagnosis; LCIS, lobular carcinoma in situ.

Our involvement with *PALB2* started with a single family (Figure 1) where the proband had developed two breast cancers, had an extensive family history of breast cancer and had a daughter who had died of cancers arising as part of Fanconi anemia. As *BRC42* was known to be a Fanconi anemia gene [15], I felt that a germline *BRC42* pathogenic variant was very likely, but we tried in vain to identify this mutation. Within months of publication of the *PALB2* discovery paper [6], we had identified a truncating pathogenic variant in *PALB2* variant in both our index patient in the first family studied and in a second family. But we did not have proof that these variants abrogated *PALB2* function. We could not do the work alone and were happy to be able to reach out to the person who had identified the gene, David Livingston (Harvard University). Thus, our initial single family had contributed in a significant way to the *PALB2*-breast cancer discovery story.

Around that time, I refocused attention on a family with what seemed to be a rather innocuous phenotype—multinodular goitre (MNG). I was particularly interested as this was the largest MNG family ever published [16] (Figure 2). In collaboration with Michael Stratton, then at the Institute of Cancer Research, Sutton (U.K.), and Steven Narod (Toronto), we showed that the gene associated with MNG in this and another Montreal family [17] was localised to 14q32, but that the linked region was large [18]. When Ashley Hill (then in St Louis) published her finding that, surprisingly, *DICER1* (a gene mapping to 14q that we had considered as a candidate but not yet sequenced) was the cause of a familial form of a rare pediatric lung tumor, pleuropulmonary blastoma (PPB) [19], we immediately sequenced the genes of these two families. We then worked hard to find other families with MNG and *DICER1*, but the real breakthrough came after reading John Priest's paper [20],

FIGURE 2. DICER1 processing of precursor microRNAs.

Figure 2. Most miRNA genes are transcribed by RNA polymerase II in the nucleus. Primary miRNAs (pri-miRNAs) are then cleaved by the microprocessor complex, formed by DROSHA and DGCR8, into precursor miRNAs (pre-miRNA), which are subsequently exported to the cytoplasm by exportin-5. In the cytoplasm, DICER1 cleaves pre-miRNAs into mature microRNAs (3p-5p duplex) and participates in the assembly of the miRNA-induced silencing complex (miRISC). Finally, the guide strand of the mature miRNA (frequently the 5p) bound by an Argonaute protein targets mRNAs for post-transcriptional gene silencing (wild type DICER1 panel). However, in cells expressing a mutant DICER1 that lacks a functional RNase IIIb catalytic domain, the maturation of 5p strand is blocked whereas the 3p strand is properly processed; therefore, only the 3p strand will be loaded into miRISC to target mRNAs (mutant DICER1 panel).

published around the same time as Hill's paper, that pointed out that MNG and the rare ovarian tumor, Sertoli-Leydig cell tumor (SLCT) were often seen in families with cases of PPB. We conjectured that there could be families that have no PPB, but instead have MNG and SLCT and that germline *DICER1* mutations would be responsible for these disorders. We scoured the literature, and noticed that this association had been reported in 1974 by Fraumeni and colleagues [21]. Fortunately, we were able to contact authors of two relevant papers and identified likely pathogenic mutations in *DICER1* in both families, as well as in a third case from Montreal. With five families, we could be sure that MNG, with or without SLCT, was part of what was later to be named DICER1 syndrome [22]. We now know that this syndrome includes many rare to very rare tumors, most of which likely result from some degree of arrested embryonal development, presumably triggered by the microRNA imbalances that are a key feature of the syndrome (Figure 2). Most of the tumours arise in infancy, childhood or adolescence; interestingly, after the age of 45 years or so, it is rare for *DICER1* pathogenic variants to be associated with tumor occurrence. More information on DICER1 syndrome can be found on our multi-lingual website, www.dicer1syndrome.ca. Our work, and the work of many others, has recently been summarized in a 10-year review of DICER1 syndrome [23].

My interest in rare ovarian tumors such as SLCT made me wonder if genes for other similarly rare familial ovarian tumors could be identified using only one or two families. One such disease is small cell carcinoma of the ovary, hypercalcemic type (SCCOHT). By this time, exome sequencing had become the technique *de jour*, so we joined in. In collaboration with a leading McGill University computational biologist, Jacek Majewski, we and three other groups identified predicted pathogenic mutations in the gene encoding the SWI-SNF chromatin remodelling protein *SMARCA4* in DNA from affected women with SCCOHT [24-27]. Our initial findings from exome sequencing in three families with more than one case of SCCOHT convinced us that we had identified the correct gene. It is one of the most lethal of all ovarian cancers, with a mean age of onset of 24 years, and there is no reliable way to detect the cancer early. Subsequent work by a number of researchers, including Sidong Huang (McGill) in collaboration with our group, has identified several plausible treatment targets [28-30]. Thus, in only five years we have moved from discovery to potential therapies. Last year, the International SCCOHT Consortium was founded (www.smallcellovarian.org/consortium.html) with the goal of accelerating research into this devastating cancer.

This collaborative effort involved one of the leading neuropathologists, Martin Hasselblatt (Münster, Germany), a recognised SMARCA4 expert, who carried out the critical immunohistochemical work in our early papers. He told us a seemingly unrelated story that he wondered if we could help with. Familial dysembryoplastic neuroepithelial tumor (DNET) is exceptionally rare, and a family known to Hasselblatt, was the best documented. The problem was that we had blood DNA from various family members, but only tiny formalin-fixed paraffin-embedded tumor tissues. Again, Majewski's team came through and, together with my post-doctoral fellow, Barbara Rivera, and Nada Jabado (McGill) and her collaborators, we were able to show that germline and somatic mutations in *FGFR1* are critical players in the etiology of this disorder. This is the first time a tumor susceptibility gene had been identified with only one family as the starting point. This paper was published back-to-back with a similar paper on somatic *FGFR1* mutations in DNETs and related tumours [31,32]. The fascinating aspect of this story is that the DNETs arising in the family all had two *FGFR1* pathogenic variants (one germline and one somatic) but in this case, the mutations are *in cis*. Again, perhaps the only example of an inherited susceptibility to tumors where the second hit has to be on the same allele. Further work to understand the critical determinants is ongoing.

The question of ovarian cancer susceptibility in *PALB2* heterozygotes remains uncertain, but it does appear that there is a slightly increased risk. The risk is insufficient, however, to explain the occurrence of ovarian cancer in *BRCA1/2*-negative families featuring both breast and ovarian cancer. In 2011, *RAD51D* was identified as a missing piece of the ovarian cancer susceptibility puzzle [33]. Eight ovarian cancer probands with predicted protein truncating variants were found among 911 breast-ovarian cancer families, compared with one in 1,060 controls. At the time, it was considered that missense variants in *RAD51D* did not confer risk of ovarian cancer. We and others soon replicated the initial finding, making *RAD51D*, along with *RAD51C*, discovered a year before [34], a new Mendelian risk factor for ovarian cancer. Around 2012, a French-Canadian family was referred to us that included three sisters with high grade serous carcinoma (HGSC) of the upper genital tract, all of which were pathologically confirmed (Figure 3). Clinical *RAD51D* testing was undertaken, and the testing lab identified a missense variant in *RAD51D*, c. 620C>T, encoding p.S207L. At the time, this variant was classified as a variant of uncertain significance, and we noted one other laboratory had reported this variant. We had difficulty contacting other family members, or obtaining

FIGURE 3. Three sisters with high grade serous carcinoma.

Figure 3. The initial *RAD51D* pedigree we studied in Montreal. Abbreviations: +, heterozygotes for c.620C>T. p.S207L; arrow, proband; ovary, ovarian HGSC; endometrium, endometrial HGSC; breast, breast cancer; intestine, intestinal cancer.

tumours, so it was difficult to take the project forward. The break came when Eva Tomiak (Ottawa) contacted us, asking our opinion on a French-Canadian family with three cases of HGSC and the same *RAD51D* variant. Initially, I thought she must be referring to the original family, but I soon realised it was a second family. This motivated me to dig deeper into the story, and again Barbara Rivera was on hand to sort the project out. After sequencing thousands of tumors, haplotyping heterozygotes and undertaking exome sequencing of ovarian tumors, we felt we had the story, but we lacked functional data to show that the missense variant was interfering with *RAD51D* function. In a virtuoso series of experiments, Alex Orthwein (McGill) proved that the variant was essentially null for *RAD51D* function. Approximately five years after we first met the family, we were able to report that this variant is the commonest single mutational cause of HGSC in French-Canadian women, accounting for approximately 4% of all cases [35], and the first *bona fide* pathogenic missense variant in *RAD51D*. Population-wide testing of this variant, along with *BRCA1/2* founder mutations identified in French Canadians [36], could make in-roads into this disease.

These five stories show that each project was self-contained, but there is a thread that runs through them all—initial clinical observation, reading of the literature, outstanding students and fellows, concerted efforts to identify key collaborators, focused pursuit of the goal and, finally, an ability to “keep the show on the road”.

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