Lecture

Refining Clinical Practice: Transforming Science Research into the Art of Medicine+

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Abstract

This article traces the development of modern day breast cancer treatment from 1896 when observations were made on the positive response of patients to oophorectomy. The oestrogen receptor was defined and tamoxifen was discovered to be an effective anti-oestrogen. The genes related to breast cancer, BRCA1 and BRCA2, were found to confer high risks of breast and ovarian cancer on women with these genes. The application of functional genomics to breast tumours would result in a more accurate classification of cancers and hopefully more specific therapy and better clinical outcomes. An important off-shoot of anti-oestrogen research has resulted in a new class of drugs called selective oestrogen receptor modulators for treatment of osteoporosis and dyslipidemia.

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Good morning fellow colleagues, ladies and gentlemen

I am humbled by the invitation given to me by Changi General Hospital to deliver this lecture at your 5th Annual Scientific Meeting with the theme "Frontiers of Medicine". Thank you very much for the honour accorded me. Your CEO, Mr Udairam, and your CMB, Prof Fock Kwong Ming, are no strangers to me. Actually, they are my very good friends.

My links with CGH go back to Thomson Road General Hospital, later renamed Toa Payoh Hospital (TPH) with the late Dr Jimmy Choo, and the late Professor Seah Cheng Siang running the Departments of Surgery and Medicine respectively. Yes, that was last century. Over 30 years ago, I was a medical student posted there for Elementary Clinics. And 30 years ago, 1973 to be exact, I started my working life as an intern there; first as a medical houseman, then a surgical houseman doing 6 months of each. In 1976, after National Service and a short stint at the Kandang Kerbau Hospital as Medical Officer, I returned to train in Medicine at TPH before being posted to SGH.

When CGH was born, transformed from TPH and relocated to this present site, I happened to be one of your Board Members serving under Chairman Mr Lee Yong Siang. It was first named Eastern General Hospital. Today, I continue links through sitting on your Research Advisory Committee giving out funds to spur and encourage research by your staff. So, I thought it appropriate to talk today on the onerous responsibility placed on clinicians to transform knowledge from the depths of science research into springs of life in the art of medicine for the benefit of our patients.

We should not be too hung up about high cost medicine. We should instead do the best we can with those within the reach of patients and ourselves. We can make the difference with what we already have.

So let us go back to the late 19th century before modern medicine was born.

The Oestrogen Story

Over 100 years ago, in 1896, Dr George Beatson[1] made the astonishing decision to treat advanced breast cancer in a premenopausal woman by removing her ovaries. This was reported in the Lancet. By 1900, Stanley Boyd[2] at Charing Cross Hospital in London had collected the records of 46 premenopausal women with breast cancer to document their responses to oophorectomy. Only 37% responded to the procedures. For the next 60 years, the reason for this remained obscure.

In 1896 when Dr Beatson made his report, the endocrine role of the ovaries was completely unknown. In 1923, Drs Allen and Dorsy[3] in St. Louis described their finding of an

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'estrus stimulating principle' in the follicular fluid of pig ovaries. The ovaries were known to control reproductive function. Their studies illustrated an important principle. The close relationship was necessary between the laboratory and medicine to unlock the mysteries of human physiology. They discovered the chemicals produced by the ovaries that were subsequently identified as circulating messengers. They were named oestrogens (estrus in Latin means frenzy to reflect the heat stage of animals). Oestrogens were found to selectively activate oestrogen target tissues (uterus, vagina, breast) in a woman. The concept of an endocrine control of one organ by a distant organ becomes an established fact.

Why did some breast tumours respond to the removal of ovarian influence whereas others did not? This clinical question is an example of how research projects start. The bench and bed are related bi-directionally. In this case, clinical observation led to further laboratory investigation. Some 40 years on, in 1962, Drs Elwood Jensen and Herb Jacobson[4] synthesized radioactive oestradiol, the most potent of the natural oestrogens in a woman's body. They injected radioactive oestradiol into immature female rats and sacrificed them at different times over the next 24 hours to find out where the oestrogen had gone. It was found bound to all tissues initially but only retained in oestrogen target tissues. They reasoned that there must be a receptor by which the target tissues retained the oestrogen.

The Oestrogen Receptor

The oestrogen receptor (ER) protein was subsequently isolated from the rat uterus in 1966 by parallel research ventures at the University of Chicago and the University of Illinois (Jensen's group and Gorski's group). The work rapidly translated to the clinic and established the concept of a pattern of oestrogen target tissues containing the ER throughout a woman's body. Jensen further reasoned that since oestrogen controls oestrogen action via the ER, then perhaps breast cancer growth was similarly regulated. In the laboratory, he established that some breast cancers did indeed contain the ER. With further clinical studies, he established the principle that patients whose tumours contained the ER would respond to an endocrine manoeuvre, but others whose tumour was ER negative would be unlikely to respond.[5] This principle was established in Bethesda, Maryland in 1974 at a National Cancer Institute-sponsored meeting. Investigators from around the world were invited to pool their clinical data on the usefulness of the ER assay to predict hormone responsive breast cancer. This conference was enormously successful and the consensus revolutionised the treatment of breast cancer.[6] A patient with an ER positive tumour has a 60% chance of responding to endocrine ablation, but a patient with an ER negative tumour has only a 10% chance of a response.

Ablation

Surgeons now had a test that could help them select women most likely to respond to endocrine ablation and therefore reduce the cost and morbidity of treating everyone with ovarian or adrenal ablation. The ER test would avoid disappointing results in two-thirds of patients. However, dramatic progress was being made in parallel research that would make endocrine ablation obsolete. There was a better way to treat patients – by blocking oestrogen action in the tumour itself. If oestrogen was the key that unlocked the growth mechanism in breast cancer, perhaps a drug that blocked the lock could be formed and ablative surgery to remove the source of hormone could be avoided.

Anti-oestrogens

In 1958, Dr Leonard Lerner[7] and others reported the unique pharmacological properties of the first non-steroidal anti-oestrogen MER (ethamoxytriphetol.) It was anti-oestrogenic in every species tested[7] and no other hormonal or anti-hormonal activity was detectable. Theoretically, an anti-oestrogen could have many uses in gynaecology or for the treatment of breast cancer. What seized the imagination of the pharmaceutical industry was neither of these. Rather, it was the discovery that an oestrogen could prevent pregnancy in laboratory animals after they had mated. MER had low potency and troublesome side effects in patients, but its successor compound, clomiphene an analogue of the oestrogen chlorotriphenylethylene, was more potent. In clinical trials,[8] rather than acting as an anti-fertility agent, clomiphene actively induced ovulation and this is its use to this day in infertile women.

Because of the link between oestrogen and the growth of some breast cancers and the observation in the laboratory that anti-oestrogen could block the binding of radioactive oestradiol in its target tissues, the rationale for clinical studies in the 1970s was strong. However, early studies were abandoned because patients suffered severe toxic side effects.

The most potent non-steroidal oestrogen, diethyl-stilbestrol, was discovered by Sir Charles Dodds in the late 1930s. Structural analogues, the triphenylethylenes were subsequently found to be potent, long-acting oestrogenic drugs. These drugs were tested by Sir Alexander Haddow[9] in his pioneering studies on the value of high-dose oestrogen therapy to treat advanced breast cancer in postmenopausal woman and to treat prostate cancer in men. This breakthrough in 1944 established a somewhat paradoxical but effective therapeutic option still used today.

Tamoxifen

By synthesizing numerous analogues of triphenylethylene systematically at ICI Pharmaceuticals (now Astra Zeneca) and testing them clinically, Drs Harper and Walpole, in the

mid-1960s, discovered that the trans isomer of a triphenylethylene ICI 46,474 was the anti-oestrogenic compound of choice, whereas, its cis geometric isomer, ICI 47,699 was an oestrogen in all tests.[10] So the trans isomer, called tamoxifen, was marketed in the 1970s for the induction of ovulation but Dr Walpole suggested using it as a treatment for breast cancer. Tamoxifen had anti-oestrogenic activity in rats and primates and it showed promising results as an anti-tumour agent in women with breast cancer. Furthermore, tamoxifen had high anti-tumour potency and virtually no side effects. By blocking the ER, it prevents oestrogen turning on all reactions necessary to instruct the cell to divide.

In 1973, tamoxifen was approved by the UK Committee on the Safety of Medicines for the treatment of breast cancer and on 30 December 1977, the US Food and Drug Administration (FDA) gave approval for its use in the treatment of advanced disease in postmenopausal women. It is the first-line endocrine therapy for the treatment of breast cancer.

Further approvals from the FDA were obtained for tamoxifen; in 1985, as adjuvant therapy with chemotherapy in postmenopausal women with node positive breast cancer; in 1986, as adjuvant tamoxifen alone in the same group of postmenopausal women with node positive breast cancer; in 1989, for use in premenopausal women with oestrogen receptor positive advanced cancer; in 1990, as adjuvant for pre and postmenopausal patients with node negative, oestrogen receptor positive breast cancer; in 1998, for the reduction of the risk of breast cancer in high-risk pre and postmenopausal women. Also, not forgetting the male with breast cancer who has poorer prognosis, the FDA approved the use of tamoxifen to treat advanced breast cancer in men in 1993.

In 1994, the FDA approved the claim that tamoxifen prolonged the overall survival of the breast cancer patient. And based on randomised adjuvant clinical trials,[11] tamoxifen is also approved for use in the reduction of contralateral breast cancer in women after a diagnosis of breast cancer.

Hence, we can learn from this experience spanning over 100 years. The first principle was that a close relationship was necessary between the laboratory and the drive to unlock the mysteries of the human physiology. The second, from concept to fact when oestrogens were found to be the means of an endocrine control of one organ (e.g. breast) by a distant organ (e.g. ovary.) The third principle was the question of how this was possible which led to the discovery of oestrogen receptors. The fourth was the pooling of clinical data leading to the analysis of the usefulness of the ER assay in predicting hormone-responsive breast cancer. These were followed by the systematic search of anti-oestrogens that could selectively block the ER and tamoxifen was discovered. And finally, there were many collaborative clinical trials involving patients with breast cancer that gave the clinical evidence for the FDA approvals for the clinical indications for usage of tamoxifen in patients. These same 6 principles are applicable today.

I move on now to cancer genetics.

Breast Cancer Genes

Age is a major breast cancer risk, with the majority occurring in women over 55 years old. Family history is the most widely recognised risk factor and it occurs at 2 levels. One is due to a genetically inherited predisposition to breast cancer and the other an increased familial incidence of breast cancer. For those with predisposing genes, mutations in 3 genes – p53, BRCA1 and BRCA2 – have been identified. Genetic breast cancer accounts for 5% to 10% of all breast cancers.

The BRCA1 gene is located on chromosome 17q 12-21 and confers an autosomal dominant susceptibility for breast cancer. Mutations of this gene are associated with a risk of both breast and ovarian cancer.[12] For women with these mutations (and more than 300 different mutations have been identified), the risk of developing breast cancer before age 50 is about 50%, increasing to 75% by age 65. The risk of a second breast cancer is 65% by age 70. The risk of ovarian cancer development is less well quantified and ranges from 20% to 50%. The BRCA1 gene is mutated at different places in the germ line. The growth of a tumour results from the loss of a normal allele. The comparative cumulative risk of breast cancer with and without this gene is 80% versus 8% by age 70. Similarly, for ovarian cancer the cumulative risk is 44% versus 0.6%.

The BRCA2 gene is located on chromosome 13q 12-13 and mutations appear to carry the same level of breast cancer risk as mutations of BRCA1.[13] Mutations are also associated with an increased risk in breast cancer in men.

The BRCA1 gene encodes 1863 amino acid RING-finger protein (220/KD) which suggests that the protein functions as a tumour suppressor in the nucleus of the cell by blocking excessive gene activation. BRCA1 may also be a secreted protein belonging to the granin family. Whatever it is, as a protein, it appears to be an inhibitory growth regulator, or brake, that is capable for preventing growth. So if it is mutated or damaged, the inhibitory function cannot be carried out and growth will become relentless. Most inherited BRCA1 mutations produce truncated proteins that vary from 5% to 99% of the full-length protein. Whereas point mutations in BRCA1 of sporadic tumour are very rare, complete somatic deletion of one allele of BRCA1 occurs in approximately 50% of sporadic breast cancers.[14]

Molecular Signatures

Cancer is characterised by genetic instability. The activation of onco-genes, the destruction of tumour suppressor genes by mutagens and the process of carcinogenesis fuelled by oestrogen can result in breast cancer. But no 2 breast cancers are the same. Each cancer has a genetic "molecular signature" unique to itself. And this can be determined using genetic analysis with microarray technology to recognise the cell type, its origin, and the maturation stage of the cell that began its malignant clone. Breast cancers, even if they were all ER positive, are still a heterogenous group of tumours.

Gene expression profiling is a genomic technique that has proved effective in deciphering this biologic and clinical diversity. The approach relies on the fact that only a fraction of the genes encoded in the genome of each cell are expressed – that is, actively transcribed into messenger RNA (mRNA). The abundance of mRNA for each gene depends on a cell's lineage and stage of differentiation, on the activity of intracellular regulatory pathways, and on the influence of extracellular stimuli. To a large extent, the complement of mRNAs in a cell dictates its complement of proteins, and consequently, gene expression is a major determinant of the biology of normal and malignant cells.

In the process of expression profiling, robotically printed DNA microarrays are used to measure the expression of tens of thousands of genes at a time; this creates a molecular profile of the RNA in a tumour sample.[15] A variety of analytic techniques are used to classify cancers on the basis of their gene expression profiles.[16,17] There are 2 general approaches. In an unsupervised approach, pattern recognition algorithms are used to identify subgroups of tumours that have related gene expression profiles. In a supervised approach, statistical methods are used to relate gene expression data and clinical data. These methods have revealed unexpected subgroups within the diagnostic categories of cancers that are based on morphology and have demonstrated that the response to therapy is dictated by multiple independent biologic features of a tumour.

What form of technology will be used for the molecular diagnosis of cancer in the future? From gene expression profiling comes 2 clear lessons. One, multiple genes need to be studied to distinguish most types of cancer, and two, quantitative measurement of molecular differences among tumours results in clinically important diagnostic and prognostic distinctions. An important goal will therefore be to develop a platform for routine clinical diagnosis that can quantitatively measure the expression of a few hundred genes. Such a diagnostic platform would allow a quick translation of what has been learnt about important molecular subgroups within each cancer. For new clinical trials, genomic scale gene expression profiling must be included in order to identify genes that influence the response to the agents under investigation. This way, the molecular diagnosis of cancer on the basis of new advances in treatment can be refined. Eventually the goal of tailored therapies for molecularly defined diseases can be reached. And this could hold true not only for breast cancers and other cancers, but also common diseases that are not malignant.

Additional Benefits of Tamoxifen

Although always referred to as an 'anti-oestrogen', tamoxifen is really an attenuated oestrogen. It acts as an anti-oestrogen to block breast cancer growth, but it also has an oestrogenic effect to help maintain bone density and to decrease circulating cholesterol. These effects of oestrogens protect younger women from osteoporosis and heart disease till after the menopause. And tamoxifen does restore this protection because whereas oestrogen stimulates all sites around a woman's body including the breast cancer, tamoxifen stimulates all the sites except the breast cancer.

Osteoporosis

Women begin to lose bone mass at about the age of 30; however, a significant acceleration of this loss (up to 5% per year) is noted after menopause.[18] It has been estimated that women can lose up to 35% of cortical bone mass and up to 50% of trabecular bone mass over their life time,[19] resulting in life time risks of hip and vertebral fractures in postmenopausal white females of up to 16% and 32%, respectively.[20] Oestrogens have been shown to greatly reduce osteoporosis in postmenopausal women. And tamoxifen, contrary to expectations, although supposedly anti-oestrogenic, was found in bone organ cultures to inhibit bone resorption.[21] Using dual photon absorptionmetry, Love et al,[22] in a randomised placebo controlled trial over 2 years of 140 postmenopausal breast cancer patients given tamoxifen, showed a significant increase in bone minimal density of their lumbar spine. And this was maintained after 5 years on tamoxifen.[23]

Selective Oestrogen Receptor Modulation

The idea of targeted anti-oestrogen was born with the discovery of target site selectivity, i.e. they are oestrogen-like at some sites, e.g. bone, but inhibitors of oestrogen action at other sites, e.g. breast and uterus. The concept was to use tamoxifen or other compounds to prevent osteoporosis in postmenopausal women but at the same time prevent breast cancer in broad groups of women without risk factors other than age. So translating these concepts to the clinic effectively meant the development of designer oestrogens.[24] Such an agent would have all the benefit of oestrogens for the postmenopausal woman but with the added advantage of preventing breast and endometrial

cancer.[25] Today, this concept is known as selective oestrogen receptor modulation and the agents are known as SERMs. Tamoxifen is the first drug in this class and the second to be clinically tested is raloxifene.

Raloxifene

Raloxifene, originally called keoxifene, was first reported by scientists at Eli Lilly, Indianapolis to be an anti-oestrogen with a high affinity for the ER. As an anti-tumour agent, it is less potent than tamoxifen, and so it was abandoned for this purpose. Tamoxifen maintains bone density in the lumbar spine,[22] neck of the femur,[26] and radius[27] but not by the same magnitude as could be expected by hormone replacement therapy. Although the increase in bone density is only by 1%, tamoxifen reduced hip fracture by 50%.[28] Based on bone data (on raloxifene) in animals,[29] clinical trials were done that demonstrated the maintenance of bone density in postmenopausal women at risk of osteoporosis.[30]

Raloxifene is today FDA-approved for the prevention of osteoporosis. At 60 mg daily, it produces a 1% to 2% increase in postmenopausal bone density and reduces fractures by about 30% to 40%. What about breast cancer prevention? Raloxifene reduced the incidence of breast cancer in low-risk women by about 50%.[31] So it appears that one drug, raloxifene, used to prevent osteoporosis may well prevent breast cancer as well. This is being tested in the STAR Trial (Study of Tamoxifen and Raloxifene), a phase III double-blind trial of postmenopausal women given either daily tamoxifen (20 mg orally) or raloxifene (60 mg orally) for 5 years with follow-up for another 2 years thereafter.

Cardiovascular Benefits

When tamoxifen emerged as a proven therapy for breast cancer, there were genuine concerns that treating women with an anti-oestrogen would adversely affect their lipid profile and lead to an increased risk of heart disease. However, analysis of studies shows this not to be so. Instead, total cholesterol decreased on average by 30% with an average decrease in low-density lipoprotein (LDL) of 19%.[32] In a randomised, double-blind study of tamoxifen against placebo, Love et al[33] noted increased synthesis of VLDL leading to increased triglyceride levels and increased lipoprotein B receptors which resulted in lower LDL levels. Analysis at 5 years supported the maintenance of decreased LDL and total cholesterol.[34] However, HDL levels usually increased by oestrogen therapy are unaffected by tamoxifen. These beneficial effects are translated into reduced cardiovascular risk via lowered cholesterol and lipoprotein (a) level.[32]

Thus far, I have traced the development of tamoxifen to treat and to prevent breast cancer in women. As the first SERM, it has also beneficial effects on the cardiovascular risk lipid factors and also weakly on bone strength. Raloxifene, the second SERM, has more direct benefits on osteoporosis – both to treat and to prevent the disease. This success story provides a model for useful research that benefits people.

Conclusion

Ladies and gentlemen, thanks for accompanying me through this lecture. The laboratory and the clinic are intertwined. Translational research is the application of research laboratory findings into clinical practice. Research does not travel in straight lines. Observations in one field of science often become major discoveries in another. Novel drugs and designer molecules will continue to be produced. Clinical trials of the future will incorporate genetic molecular profiles of patients with seemingly similar diseases yet different molecular signatures. That is, what appears to be homogenous groups of patients may yet be heterogeneous at the molecular level. Identifying chromosomal and gene mutations alone may be insufficient. What counts finally is the gene expression on cells because this regulates its functions and interactions within the internal milieu and with the external environment. With these advancing technologies, the hope for tomorrow is more refinement in the art of medicine.

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