Volume 59 / June 30th, 2022

TISSUEPAPER

Histotechnology Group of Queensland

President's Report- Mark Bromley

Welcome to the second 2022 edition of TissuePaper.

Apparently that pesky La Niña has finally left our shores, taking with it the threat of more rain. Let's hope it remains that way for a while and we can all dry out!

Since our last newsletter, we enjoyed an excellent presentation by Dr Rohan Lourie on p53 along with a lab tour of Mater Pathology. It's great that we seem to be finally able to put Covid behind us and regularly have face to face scientific meetings, although I do believe we had a bit of an outbreak at the DIHC meeting in Tweed Heads where the Covid love was spread about a bit. I hope all those affected by it were not overly so.

For the diary is the upcoming trivia night on Friday 24th of June at the Normanby Hotel. Hopefully your table is booked and your brains are in high gear for an evening of fun and frolics. Following this is the joint AIMS/HGQ scientific meeting in the usual Pineapple Hotel venue on Thursday 18th August, with the topic of the respiratory system, and the Queensland State Histology Conference and HGQ AGM in The Pullman Reef Hotel in Cairns on August 7th to 9th.

So, until the next edition, enjoy the fabulous winter sun, unless you're one of our interstate readers, in which case stay warm!

In This Top Issue:

IHC AND ISH INSTRUMENTS & SOLUTIONS

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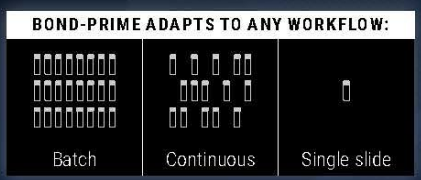

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Histotechnology Group of Queensland

By Emma Hughes
Laboratory Technician SNP, HGQ Committee Member.

On the 19th May the 2nd Scientific Meeting for 2022 was held at Mater Pathology. The event was sponsored by Metagene.

Attendees arrived first at the Brewhouse down the road from Mater Pathology for Drinks and Appetisers before heading down to Mater Pathology.

Once everyone had arrived and signed in two separate groups were taken on a Lab tour of Mater Pathology.

Figure 1: Brewhouse

Figure 2: Mater Pathology, Brisbane

Figure 3: Complex Dissection Bay

Figure 4: Specimen Storage (Processing Room)

Figure 5: Embedding Stations

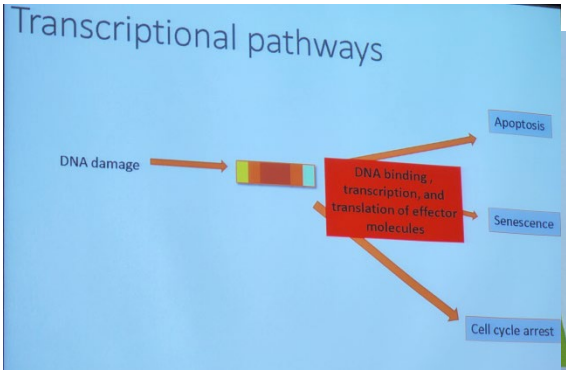
Figure 6: Microtomy Stations

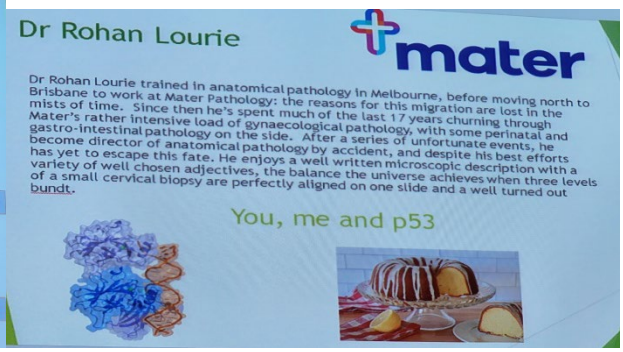
Figure 7: Special Stains

Figure 8: IHC area

After the tours were completed everyone headed into the Meeting room located next to the Histology Lab for a talk given by **Dr Rohan Laurie.** Dr Rohan Laurie is the Director of Anatomical Pathology for Mater Pathology and a renowned Gynaecological Pathologist.

Dr Laurie gave a fantastic and informative talk on the p53 protein, and its function in the human body. Why mutation in the p53 protein can be so deleterious and its involvement in many cancers.

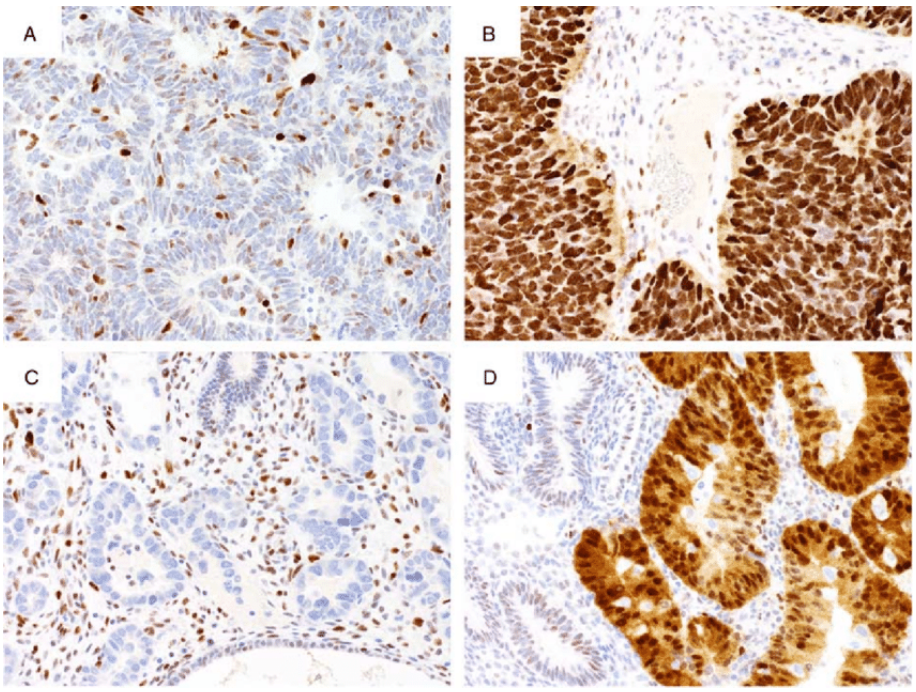




The p53 protein is involved in the transcriptional pathways of DNA replication and DNA Damage. If DNA is damaged i.e. mutations are detected, then p53 plays an important role in the management of these damaged cells. This can be done in three main ways, Apoptosis, Senescence, and Cell Cycle Arrest. Apoptosis is the process of programmed cell death. Senescence is a stable cell cycle arrest, where the cell stops dividing. This aids in the reduction of the production of more DNA damaged cells and the production of Cancer Cells.

Thus, if the p53 protein itself is damaged then the Transcription pathway will not function properly causing an increase in cancer and a worse prognosis for p53+ve cancers. Depending on the mutation of this protein there can be an over or under expression of the protein.

Mutation in the p53 protein is responsible for 50% of Glioma's, 67% of Lung Cancer's, 60% of Pancreas Adenocarcinoma's and 53% of Colorectal Cacinoma's, 50% Ovarian cancer and the frequency of p53 mutation in type I endometrial cancer is about 10–40%, whereas that in a type II endometrial cancer is about 90% [55]. Multiple cases studies were shown during Dr Laurie's presentation showing the different p53 staining patterns and how important the staining pattern can be in the correct diagnosis.



Different patterns of p53 expression. (A) Endometrial endometrioid carcinoma showing normal wild-type (non p53 mutation) pattern of p53 expression with variable proportion of tumor cell nuclei staining with variable intensity. (B) Endometrial endometrioid carcinoma, grade 3, with overexpression, showing strong staining in

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virtually all tumor cell nuclei, much stronger compared with the internal control of fibroblasts in the center. Note, there is some cytoplasmic background indicating that this staining is quite strong but this should not be interpreted as abnormal cytoplasmic pattern. (C) Endometrial serous carcinoma showing complete absence of p53 expression with internal control showing moderate to strong but variable staining. Note, wild-type pattern in normal atrophic glands at 12 and 6 o'clock. (D) Endometrial endometrioid carcinoma showing cytoplasmic p53 expression with internal control (stroma and normal endometrial glands) showing nuclear wild-type pattern. The cytoplasmic pattern is accompanied by nuclear staining of similar intensity.

Summary:

P53 Immunohistochemistry provides a direct link from mutation to immunohistochemistry to diagnosis and prognosis.

IHC contributes but is dependent on:

Well-fixed Specimens and the selection of the most relevant Block.
Good quality staining with slide and internal controls optimized to a reliable normal level.
Adequate knowledge of staining patterns to judge whether an underlying p53 mutation is likely.
Interpretation of the IHC result regarding a patient.

Thank you everyone who attended it was a great turnout and a great night. Thanks again to Metagene for sponsoring this Event and Dr Rohan Laurie and the Staff of Mater Pathology for hosting the event.
Hope to see everyone at the joint HGQ/Aims Scientific Meeting on the 25th August.

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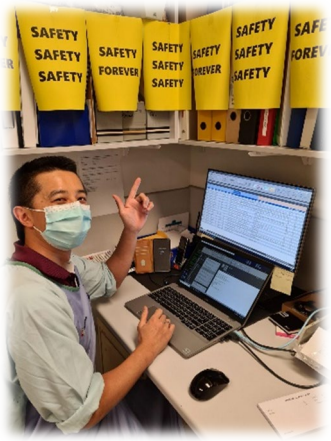
Get to know your histology team members!



Often found in the depths of the anatomical pathology departments, the work horse of any pathology laboratory, scientists/technicians and laboratory assistance in histopathology are the best of the best. Get to know one of the scientists at the Royal Brisbane and Women's Hospital – Galvin Young!

How long have you worked in Histology? I moved to Brisbane in 2008 for this Histology job, my first job out of University. Still love working here.

What are your Qualifications? Masters in Anatomy & Structural Biology with Distinction, from the University of Otago.

Tell us about your family. Born in the mighty Manawatu and left home when I was 13yo to attend boarding school in Wanganui. Lived it up in Dunedin for Uni where I met your mother… I mean a girl who later became my wife. I am the eldest of 3 boys, my wife is the eldest of 3 girls... now we have 3 gorgeous boys of our own. Most of our families are still in NZ so we try to travel and take the boys back as often as we can.

What is something you wish you were told about working in a Lab?
You will get no sun and you're not allowed to eat in the lab.

What's your ideal Holiday Destination?
Either chilling on a tropical island or snowboarding in Queenstown and eating Fergburger with a Speights.

What are your Hobbies outside the lab?
I like eating, sleeping, shooting zombies, playing touch footy and watching my boys play sport. I try coaching them but they don't listen to me.

Favourite food?
All-You-Can-Eat is the best!!…. but being more specific would be wings and ribs. Oh, and Yum Cha and bubble tea. With orange & chocolate chip ice cream and ambrosia for dessert… Then we're ready for lunch.

Most rewarding thing about working in a Histology Lab?
Knowing I'm making a difference by doing a small part, in the grand scheme of things, to contribute to the confirmation of someone's diagnosis. A good or bad result is better than not knowing why something is wrong. And Histology gets me close to the action.

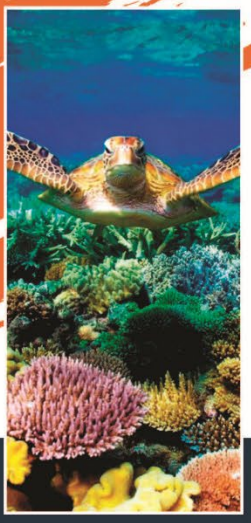
Favourite sporting team?
The All Blacks. Every kid in NZ dreams about becoming an All Black (just to beat the Wallabies). Gave it a good crack but at the end of the day science offered me a better contract.

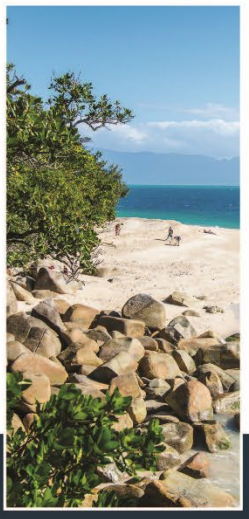
What's your favourite special stain?
A clean Periodic Acid Schiff-Methenamine (PASM) stain on a thinly cut renal biopsy section showing clean/crisp glomerular basement membrane with an H&E counter stain just brings me joy.

What's your favourite Pokemon?
Mega Slowbro... thought the name sounded sweet as when I Google'd it.

Got a funny joke for us?
What's the difference between a snowman and a snow-woman? Snowballs.
While at home in isolation I noticed a woman sitting in my living room. I started talking to her and discovered that we had a lot in common. It turns out she was my wife.

All Welcome!





2022

Hosted by the Histotechnology Group of Queensland Incorporated (HGQ), the conference is a great opportunity to learn and network among some of the most knowledgeable people in Queensland on histology.

The Committee is eager for the Queensland State Histology Conference (QSHC) 2022 to be filled with technical presentations, learning of new products and services from sponsors and exhibitors, as well as the all-important networking activities.

Why Attend?

- Reconnecting
- Networking Events
- Technical Programme
- Learning

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PROGRAMME

Friday 7 October

Pre-Conference Workshop (Dry)	1.00pm – 5.00pm
Welcome Networking Function	5.30pm – 7.30pm

Saturday 8 October

Conference Sessions	9.00am – 5.00pm
Conference Dinner	6.30pm – 10.30pm

Sunday 9 October

Conference Sessions	9.00am – 2.00pm

(Includes Networking Brunch and HGQ AGM)

NETWORKING PROGRAMME

Welcome Networking Function

Friday 7 October 5.30pm – 7.30pm
Held within Exhibition Area

Conference Dinner

Saturday 8 October 6.30pm – 10.30pm
Cairns International Hotel
(across the road from Pullman Reef Hotel Casino, Cairns)

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Back to basics: Anatomical Pathology at RBWH

Do you remember when you first started your journey in the wonderful world of histology? In this edition of 'The Tissue Paper' we go back to the beginning and learn about a student experience while on placement at the Royal Brisbane Women's Hospital. Griffith University student **Steve Ng** tells us about his time on placement under the supervision of Supervising Scientist (and HGQ committee member!) **Christopher Boyle**.

Student lab floating experience

A long awaited first morning on placement had come to a crossroad with the access door secured and no way to get in. There was a phone on the wall though but what number to dial?

Well, if you had "Passion" nothing could stop you! A lab tour was conducted with all the basic principles of laboratory safety rules, fire plan & evacuation, first aid, waste disposal, blah blah blah and here comes the best part; photo taken for making staff ID. How to do it? Find a wall cleared of obstacle or signages, get your phone ready, stand straight, and cheers!

The plan was to commence at specimen reception and get to know the AusLab.

What is AusLab?

A system that owned by ASX-listed Citadel Group (ASX:CGL) @ average $15/share. It was a conglomerate service and software entity which specialises in data managing system, IT projects and services. Its subsidiary Citadel Health is part of the Citadel Group which provide services to Health, national Security, Defence, Education and all levels of Government.

In 2020, Pacific Equity Partners (PEP) has offered to buy software and services company, The Citadel Group (CGL), for $503 million. PEP is a leading Australian based private equity firm and has over $5 billion worth of assets. The companies had signed a binding Scheme Implementation Deed (SID), under which PEP will purchase 100 per cent of Citadel shares. However, this deal does need shareholder approval. PEP has offered Citadel shareholders $5.70 per share in cash, valuing Citadel's equity at $448.6 million

AusLab had been decades-long used by Queensland Health. It was also used by NSW Health Pathology and several large health services in Victoria.

I would say the AusLab manual was extensive and informative. User interface was in matrix format and hot keys were golden if you know how.

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The Art of Grossing - Cut-up

Human specimens were categorised on benches, pending for artist registrars to hand pick and perfect their craftmanship.

Gross examination, frequently called “cut-up”, was a fine art that was quite frequently disregarded as just a boorish demonstration - however there was esteem in it. There was magnificence in something as straightforward as a stroke of the surgical blade sharp edge or the flick of a utensil as ink was applied to the edge of the resection edge, halting barely shy of the mucosa. With that equivalent upward light sparkling off the surgical tool, a specialist craftsman could utilize these sharp edges with such fine accuracy as to cut a piece of tissue a couple of millimetres thick, yet keep up with every one of the suitable edges and designs.

To these specialists, the surgical tool edge was an expansion of themselves. This edge took into consideration the exact, yet complex control of multi-organ resections that, to outcasts, might give off an impression of being just an indistinct piece of tissue.

Tissue Processing

It portrays the means expected to take a creature or human tissue from obsession to the state where it was totally penetrated with a reasonable histological wax and were ready to embed for section cutting on the microtome.

Tissue handling could be performed physically, yet where numerous examples should be managed, it was more helpful and significantly more effective to utilize a computerized tissue processor

There were two principal kinds of processors: plunge and dunk machines were examples where the samples moved from one holder to another to be handled, and the liquid exchange types where examples were held in a solitary cycle chamber or counter and liquids were siphoned in and out as required. Most current liquid exchange processors utilize raised temperatures, powerful liquid flow and integrate vacuum/pressure cycles to upgrade handling and decrease handling times.

Microtomy

The goal of this progression is to cut 2-3 µm thick segments from paraffin blocks. This is accomplished utilizing accuracy blades (microtomes). To get consistent top notch and incredibly flimsy tissue segments, expendable cutting edges ought to be utilized and changed after a set number of blocks or when harmed by little bits of bone inside the tissue for instance. The paraffin block is mounted on the microtome holder. Segments are cut as a lace and are floated on a water bath kept at 42 °C to extend the paraffin segment.

A microscope glass slide is set under the chosen tissue segment and eliminated from the water bath. Tissue segments are then permitted to dry, ideally in an oven at 65°C.

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Case study

By Steven Ng
Acknowledgement: Dr Fiona Tang MBBS, FRCPA, Pathology Queensland - Central, RBWH

A 66-year-old female with history of Lynch Syndrome was having biopsy at dermatology clinic.

Three shave biopsy of skin specimens were taken from:

Right tip of nose

Right occiput superior – tan papule on skin surface

Right occiput inferior – tan macule on skin surface

Microscopy indicated an excoriated sebaceoma as shown in Figure 1 (H&E @ 40X).

Sebaceoma is a benign yellow or flesh coloured lesion origination from sebaceous gland in the skin. It is commonly found on the face and neck.

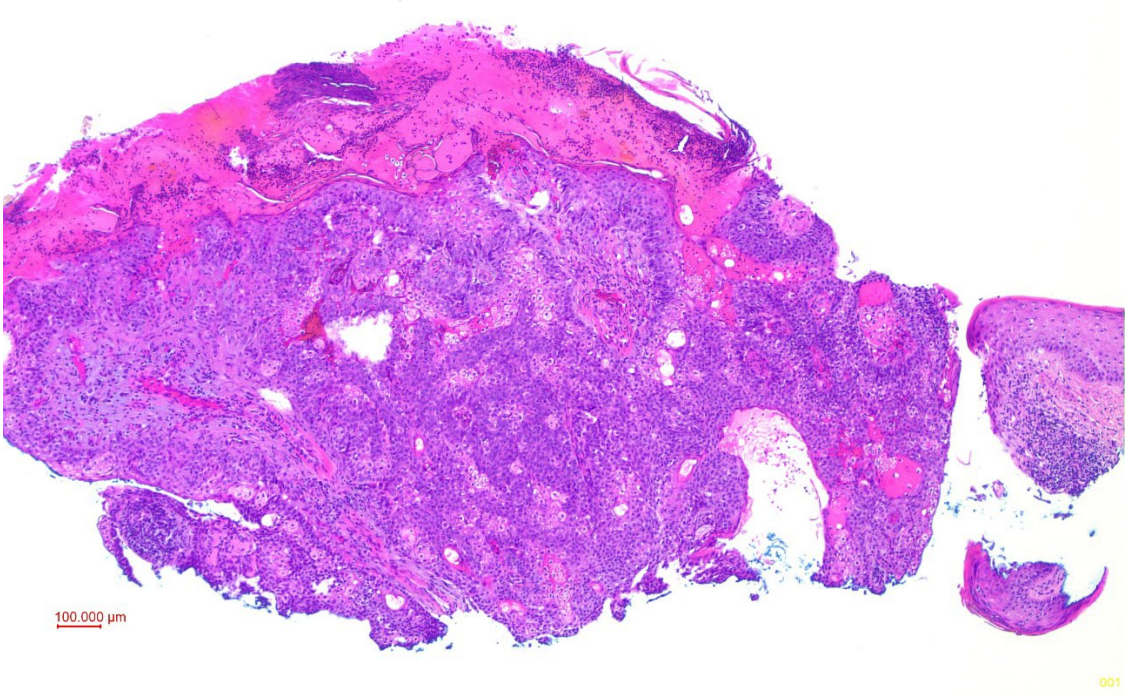

Figure 9: H&E stained slide of the case

Sebaceous glands are epidermal appendage associated with the upper portion of hair follicles. Sebocytes are the major cell type in the sebaceous gland.
In figure 2 (H&E @ 40X) marking a sebocyte with healthy nuclei. Growing cells were mitotically active. They accumulate sebum in unstained droplets which fill the cytoplasm. As they mature, they were moving outward toward the lumen of hair follicle. When sebaceous cells die its nuclei became pyknotic with intense basophilic.

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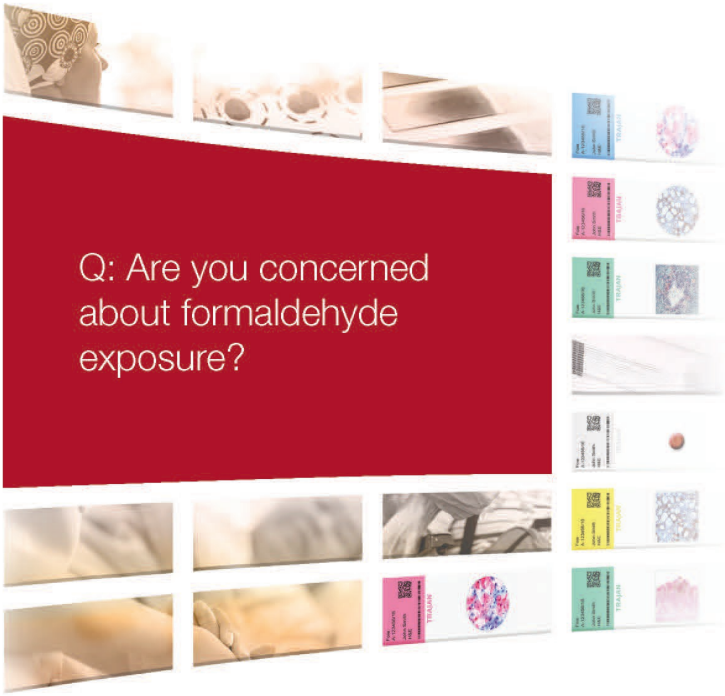

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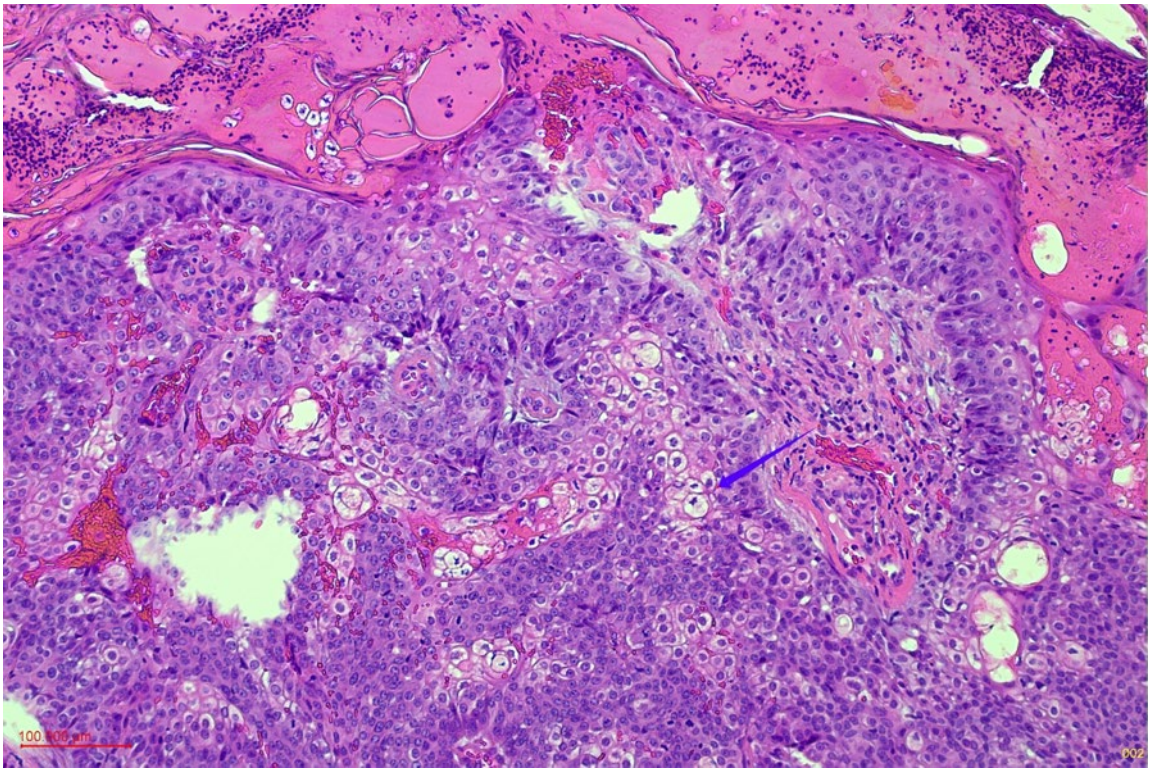
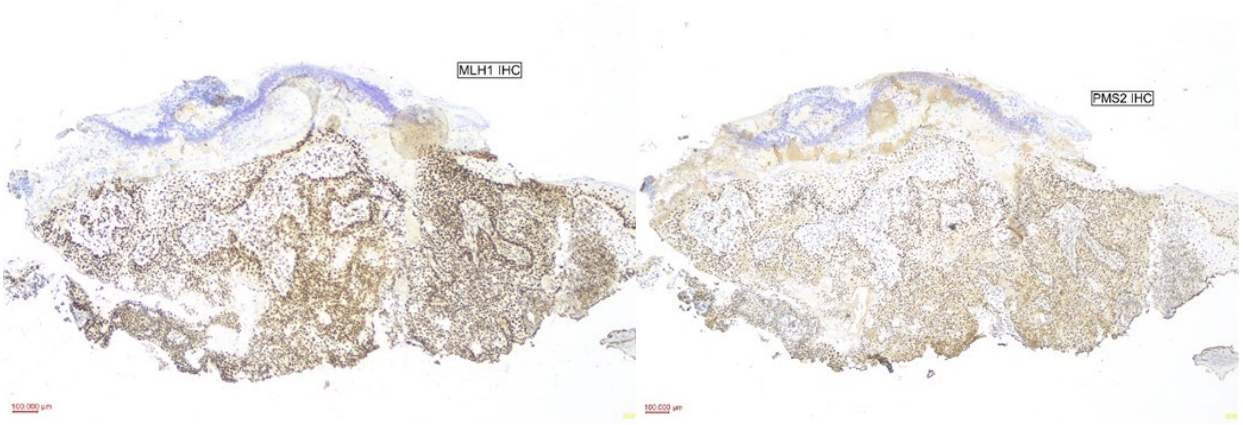


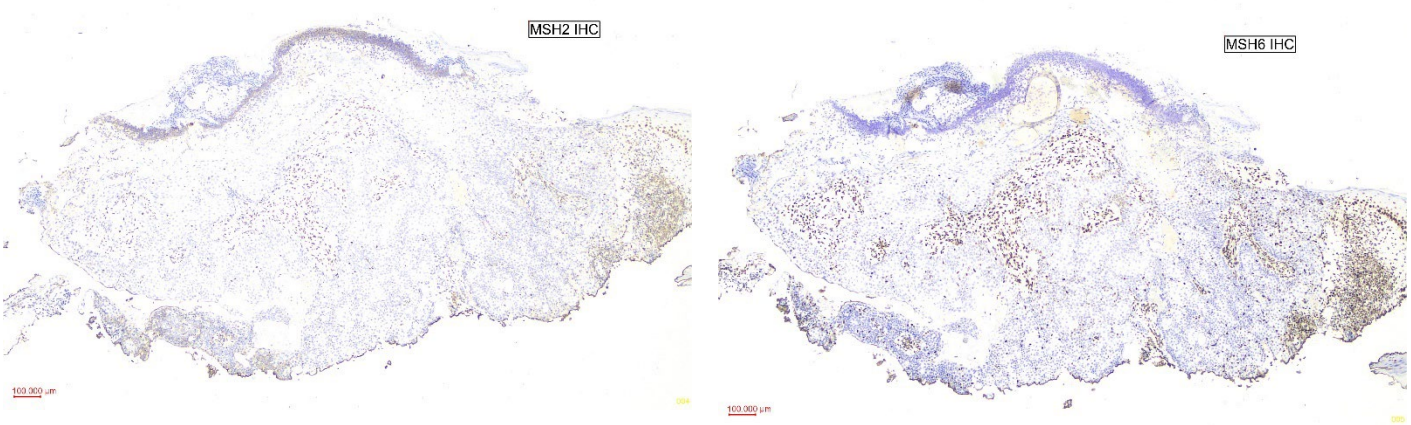
Figure 10: Seboctyes (blue arrow) with healthy nuclei and clear cytoplasm. They accumulate sebum as unstained droplets which fill the cytoplasm. As they mature, they move toward the lumen of the hair follicle.

Immunohistochemistry for mismatch repair gene products has been performed on paraffin block as shown (IHC @ 40X):

MLH1 and PMS2: Retained (positive) nuclear reactivity within tumour cells.



MSH2 and MSH6: Loss of nuclear reactivity within tumour cells.



In Summary:

The patient was diagnosed with sebaceoma; MSH2 and MSH6 MMR (mismatch repair protein) deficiency in keeping with "Muir-Torre Syndrome"

What is MTS?

Muir-Torre syndrome (MTS) is a form of "Lynch syndrome" and was characterized by sebaceous (oil gland) skin tumors in association with internal cancers. The common site involved is the gastrointestinal tract, with >50% of affected having colorectal cancer. Skin lesions may develop before or after the onset of the internal cancer.

MTS is a rare inherited disorder. It is defined by the occurrence of sebaceous neoplasms including adenoma, sebaceoma/epithelioma or carcinoma, +/- keratoacanthoma, and visceral malignancies.

MTS is an autosomal dominant disorder due to mutations in the DNA repair genes hMSH2 and hMLH1. The severity of MTS expression varies among family members.

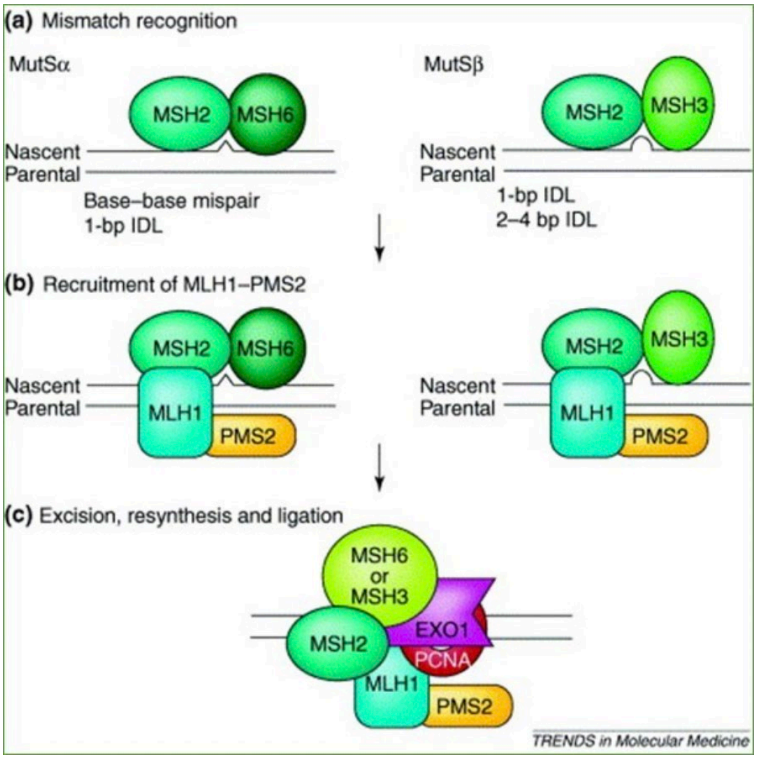
Treatment for MTS is multidisciplinary and genetic counselling and follow up screening tests, e.g. colonoscopy, pelvic and urinary examination would be recommended.

What is Lynch Syndrome?

Also known as HNPCC (Hereditary nonpolyposis colorectal cancer) is an acquired hereditary mutation which allows individuals an expanded opportunity of fostering specific diseases across their lifetime, frequently at a more youthful age than everyone <50 years old.

It is caused by a mutation in one of the body's DNA Mismatch Repair (MMR) genes. These genes are:

MLH1, MSH2, MSH6, and PMS2



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MMR genes, heterodimer complexes and their role in MMR pathway. MSH2 and its partner (MSH6/MSH3) recognizes DNA mismatch (A). Recruitment of MLH1 and its partner PMS2 and other cofactors (PCNA) and enzymes (EXO1) conduct excision, resynthesise and ligation of the repaired DNA strand (B & C) (Wei et al., 2002).

While working accurately, these qualities work by fixing the errors that can happen when DNA is duplicated in anticipation of cell division. In Lynch condition, an individual acquires one working duplicate and one non-working duplicate of one of these genes. While non-working MMR genes neglect to fix these errors in DNA, blunders collect, which might prompt uncontrolled development of cells and in the long run might become cancer.

Future events:

Date: 18th August
AIMS Joint Meeting
Venue: TBA

Date: 7th – 9th Oct
Queensland State Histology Conference and HGQ AGM
Venue: Pullman Reef Hotel Casino, Cairns

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