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NEWS

In this issue

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What does Virchows Archiv offer this month to its readers? To begin with, Chiu et al. (https://doi.org/10.1007/s00428-019-02557-1) explore the pattern of SOX10 immunoreactivity in breast cancer. The protein, which is involved in shuttling of molecules between the nucleus and the cytoplasm, was found originally to be expressed in neural crest derivatives but - much as it goes with immunohistochemical markers - its expression pattern over time appeared less specific than originally assumed. Knowing that SOX10 is expressed in myoepithelial cells, the authors set out to map its staining patterns in subtypes of ductal carcinoma (*in situ*) and normal breast tissue. Expression in breast cancer was found to be rare but, interestingly, almost all positive cases were grade 3 triple negative carcinomas and the sole positive *in situ* ductal carcinoma occurred in a patient who also had a SOX10 positive invasive ductal carcinoma. In normal breast tissue, not only myoepithelial cells but also luminal cells stained and moreover not all myoepithelial cells identified by other markers were SOX10 positive. The authors conclude that SOX10 cannot be regarded as a reliable marker of myoepithelial cells in breast lesions.

The cover image is from this paper and shows the pattern of SOX10 expression in normal breast tissue.

The following three papers have a strong methodological orientation. Next generation sequencing is rapidly becoming mainstream in molecular analysis of genome alterations in cancer, as it allows identification of actionable sequence variants but increasingly also copy number alterations, of which gains (equalling amplification) might be actionable. Much attention has been paid to standards in reporting of sequence variants, but this is less the case for copy number alterations. Ligtenberg et al. (https://doi.org/10.1007/s00428-019-02555-3) address this issue in an effort to develop guidelines for next generation sequencing (NGS) based copy number alteration reporting. The proposed guidelines emphasize the importance of standardized use of relative coverage (also known as 'fold-change') and B-allele frequencies (the frequency of variant alleles) in such reports. Important elements in the reports need to be statistical evidence of significance of any particular finding, evidence of the sensitivity of the used assay and limitations inherent in NGS based copy number assessments. If of high quality, these assays provide reliable information on therapeutically relevant gene amplifications, or, in case of insufficiently convincing results, indications to perform additional *in situ* assays.

What is known as 'liquid biopsies', analysis of blood samples for molecular abnormalities derived from a resident cancer, has gained immensely in importance over the last couple of years. Abnormalities can be detected in cell free circulating tumor DNA or RNA but also in circulating cancer cells or in exosomes released by cancer cells. For a variety of reasons cell free DNA (cfDNA) has gained the most momentum as its analysis (provided that the assays are sufficiently sensitive) can guide treatment decisions, notably when a profile of gene aberrations from the primary tumor is not available. In addition, emergence of additional gene aberrations during treatment can be detected, which might shed light on emerging therapy resistance. It also allows early detection of residual or recurrent disease. All of this is only relevant when the information obtained is reliable and of high quality. Deans et al. (https://doi.org/10.1007/s00428-019-02571-3) report on a meeting of leaders in this field in Europe, in which questions pertinent to standardization and quality assurance were addressed. These include rather down to earth issues such as how to draw the blood sample, plasma preparation, sample transportation, processing and storage, cfDNA extraction but also methods of mutation analysis, and interpretation and reporting of results. Evidence-based guidelines in this field are still under development, to which this paper contributes important impulses.

The increasing importance of the immune response to cancer as an indicator of the likelihood of the response to immune checkpoint inhibiting therapies provides the background for the study reported by Tahkola et al. (https://doi.org/10.1007/s00428-019-02549-1). They tackled the question if, for establishment of an 'immune cell score' as a quantitative parameter for anti-tumor activity in pancreatic carcinoma, an approach limited to 'hot spots' provided results equally clinically useful than cell counting on full sections. Digital image analysis of CD3 and CD8 stained sections

was used to count immune cell densities in whole sections or in hot spots only. It turns out that an immune cell count by hot spot counting globally provides results similar to whole section counting. However, associations of survival with immune cell count (the higher the count the better the prognosis) were stronger for whole section counts than for hot spot counts. The paper highlights two important notions. Firstly, with the increasing availability of equipment to prepare digital slides, which are the starting point for such quantifying image analysis activities, quantitative tissue parameters should be established by digital machine-based approaches rather than by (semi)quantitative visual assessment. Secondly, selection bias introduced through non-standardized selection procedures might obscure or reduce the credibility of relevant information.

Finally, a nice example of how careful observation of diagnostic case material may contribute to better understanding of molecular mechanisms involved in oncogenesis is provided in the paper by Myiai et al. (https://doi.org/10.1007/s00428-019-02560-6). The group had previously found in a fluorescence *in situ* hybridization study more frequent loss of heterozygosity in the seminoma component of mixed tumors than in pure seminoma and proposed that chromosomal instability might be involved in progression of germ cell neoplasia *in situ* to seminoma, and to embryonal carcinoma in mixed-type testicular germ cell tumors. Similar studies on a larger cohort of mixed germ cell tumors showed along this line of progression allele copy numbers to increase and more strongly deviate from the normal (modal) number. The data strongly support an important role of chromosomal instability in the molecular pathogenesis of mixed germ cell tumors.