Transcriptome analysis of endocrine tumors: Clinical perspectives
Perspectives cliniques de l’utilisation du transcriptome dans les tumeurs endocrines
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Abstract
There is considerable interest in the application of DNA microarrays to the pathologic evaluation of endocrine neoplasms. Improvements in tumor classification and prognostication, prediction of response to therapy, and comprehensive assessment of tumoral hormone production represent the major anticipated benefits. Here, some of the microarray studies that support the clinical use of transcriptome profiling for endocrine tumors are reviewed. In addition, some of the barriers to clinical implementation are discussed.

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1. Introduction
Since the development of DNA microarrays as a tool to comprehensively monitor gene expression in 1995 [1] and their application to tumors of all kinds in the ensuing decade (for a recent review, see [2]), significant effort has been applied to tumors of the endocrine system. As a result of the success of these studies, there is growing and considerable interest in microarrays as clinically relevant tools for the evaluation of endocrine tumors. The rationale for this interest lies in the following general areas: (1) improved tumor classification, (2) improved tumor prognostication, (3) prediction of response to therapy, and (4) comprehensive hormonal assessment. Here, some of the studies that support the view that microarray analysis will be beneficial to patients with endocrine tumors are reviewed. Some of the obstacles to implementation are also presented.

2. Improved tumor classification and diagnosis
It can be easily argued that endocrine tumor classification stands to benefit from the availability of more objective and reproducible classification methods. While histopathologic assessment of a tumor based on its morphology is informative in most cases and certainly will continue to be the mainstay of tumor classification [3], there are many endocrine tumors for which such assessment is clearly insufficient. For instance, pheochromocytomas, paragangliomas and pancreatic endocrine tumors are typically diagnosed in such a noncommittal fashion that no specific statement regarding the malignant potential of a specific tumor is rendered. In these instances, these tumors are presumed to be benign and cured by surgery. However, if a pheochromocytoma, for example, is subsequently proven to be malignant by the subsequent development of a metastasis, it is not considered a diagnostic mishap. Rather, it is accepted that identifying malignant tumors is not readily possible. It is anticipated that microarray analysis can either separate those truly benign tumors from benign-appearing tumors that actually possess malignant potential, or convincingly determine that all such tumors are, in fact, pathologically malignant, that is possess the ability to invade and metastasize. Time and many experiments will eventually identify the correct scenario. Furthermore, it has been well documented that significant interobserver variability regarding thyroid tumor classification exists amongst pathologists [3]. It is anticipated that microarray analysis will form the foundation for more objective evaluation of thyroid nodules [4].

The majority of microarray work done on endocrine tumor classification has been performed on follicular cell thyroid tumors. This is not unexpected, given the difficulties associated
with the diagnosis of some thyroid tumors, especially follicular-patterned tumors [3]. Many of these studies have focused on using genome-wide gene expression with the intent of developing a novel diagnostic approach. While none of these have yet been adequately validated to endorse clinical implementation, it is worthwhile to closely examine a select group of these studies to obtain a sense of what is possible and to identify what remains to be performed.

There have been at least 16 published studies that have primarily focused on using transcriptomic data for thyroid tumor diagnosis and classification [5–20]. Some studies have focused on the distinction of benign and malignant tumors, an approach that would be useful if implemented preoperatively using fine-needle aspirations (FNAs) samples. Other studies have evaluated specific tumor types, such as papillary carcinoma (PC) and follicular carcinoma (FC). Still other studies have examined a specific tumor type with different genetics mutations. The overall clinical applicability of using gene expression profiling has been discussed, with a prediction of clinical implementation of preoperative gene expression profiling in the coming years [4].

Discussion of all of the diagnostically-oriented thyroid studies is beyond the scope of this review. However, a few studies are presented to highlight the potential and limitations of this approach. Mazzanti et al. [8] profiled 71 thyroid tumors (classical PC, follicular variant of PC (FVPC), follicular adenoma (FA), and hyperplastic nodules (HN)) using cDNA microarrays. Principal component analysis revealed a global organization of tumors based on diagnosis, a result that bestows support for the quality of the gene expression data. By comparing the benign tumors (FA plus HN) to the PCs, they derived a set of 47 genes and used them to construct a diagnostic predictor model that accurately predicted the diagnosis of the tumors using a leave-one-out cross-validation method. Despite a lack of genotyping data for usual PC mutations and the lack of FCs, this study was one of the first to highlight the potential of gene expression profiles as a diagnostic tool for thyroid tumors.

Several studies have focused on the pathologically-challenging distinction of FA and FC. Barden et al. [5] generated gene expression profiles for a group of 17 FAs and FCs and identified a set of 105 differentially expressed genes. Using hierarchical clustering as a classifier, five tumors (a small testing set) were correctly classified. Likewise, Weber et al. [13], using a slightly larger set of 24 FAs and FCs, derived a set of 80 genes that were differentially expressed between the groups. From this set of genes, three were selected and then their performance evaluated as a gene expression classifier using an independent set of 31 tumors. The results were encouraging with a high sensitivity, specificity and accuracy, and await additional validation.

Gene expression profiles have also been useful for predicting the presence of mutation in a given tumor. Mutation-specific profiles for PCs with RAS, BRAF and RET/PTC mutations were first defined and then used to accurately predict the mutational status of 40 PCs by leave-one-out cross-validation using as few as one gene per mutation class [10]. As expected, one of the genes whose expression was most correlated with RET/PTC rearrangement was RET.

A recent study [19] using cDNA microarrays specifically addressed the issue of diagnostically-challenging follicular-patterned tumors; those sometimes diagnosed as a tumor with “uncertain malignant potential.” This term, as well as others, has been proposed for these tumors [21], although it has not been widely accepted due to its equivocal nature. Nevertheless, this study aptly focused on tumors that had tumors with uncertain or intermediate histological features. Comparing gene expression in 10 of these tumors (designated T-UM) to gene expression in tumors with unequivocal histological diagnoses (PC, FVPC, FTC, and FTA), the T-UMs were separated into benign and malignant groups. This study, although it tested a small group of T-UMs, provides support for the goal of developing objective pathological parameters for follicular patterned thyroid tumors.

The essential aim of much of the microarray work on thyroid tumors is to improve the performance of preoperative thyroid FNA. Using 22 FNA specimens of thyroid nodules and a hierarchical clustering-based classifier derived expression data for 25 selected genes, Lubitz et al. [16] demonstrated that diagnosis prediction of FNA samples was possible and accurate in most cases. Despite the small validation group and the need for a more robust statistical classifier, this study demonstrates the promise of gene expression profiling to improve the preoperative evaluation of thyroid nodules.

Considerably fewer studies have been performed on tumors of the adrenal gland. However, the field of adrenal pathology does stand to benefit from an intelligent application of microarrays. One of the first such studies identified a set of genes that were distinctly expressed in benign (adrenocortical adenoma, ACA) and malignant (adrenocortical carcinoma, ACA) adrenal tumors [22]. Using gene expression data from almost 3000 variably expressed genes, it was possible to separate ACA from ACA and even identify low-grade ACC. Subsequent studies have extended these results with larger cohorts but with more limited microarrays [23]. Further, an unpublished study from my lab also correctly classified a large cohort of adrenocortical tumors and may have performed slightly superior to morphologic assessment. Collectively, the few studies of adrenocortical tumors have illustrated significant potential to correctly diagnose and grade these sometimes-challenging tumors.

Most of the microarray studies of anterior pituitary tumors have focused on elucidating pathogenesis via understanding signaling rather than tumor classification [24–31]. This reflects the facts that advances in understanding pituitary tumor pathogenesis are currently needed more than diagnostic tools and that these tumors rarely evolve into carcinomas. Nonetheless, it is easy to envision a time when microarray analysis would be used to determine type of pituitary adenoma (i.e. define hormone production) and also be used to highlight those tumors at high risk for recurrence.

Two studies have specifically used microarray analysis to examine gene expression in parathyroid tissues [32,33]. The first study by Morrison et al. [33] examined parathyroid adenomas, multiple gland neoplasia, primary hyperplasia, secondary hyperplasia and normal parathyroid. Each class of tissue was associated with distinct pattern of gene expression with clear
separation of adenoma (single gland disease) from hyperplasia (multiple gland disease). Also importantly for tumor classification, the adenoma samples were divisible into two groups, type 1 and type 2 adenomas. This study clearly illustrated the potential of microarray analysis to correctly classify parathyroid pathologies.

The second parathyroid related study by Forsberg et al. [32] used unsupervised hierarchical clustering to classify a small group of sporadic parathyroid adenomas into two groups designated group I and II that correlated with underlying somatic deletion of chromosome 11q13. A set of 85 differentially expressed genes, about half of which were located at or near 11q13, were identified between the two groups. One the most differentially expressed genes was ENCI, a known Wnt pathway target gene, suggesting that activation of this pathway occurs in a subset of sporadic parathyroid adenomas. While this study cohort was too small to permit clinicopathologic correlation, the results generally support the concept that microarray analysis will provide clinically relevant information.

3. Improved tumor prognostication

A few microarray studies have examined gene expression across the histologic progression of thyroid cancer [34]. However, no studies with the primary goal of identifying those well-differentiated thyroid tumors at high risk for recurrence or metastasis have been performed. These studies will be difficult to perform, as long-term follow-up will be required given the long latency period sometimes seen with some well-differentiated thyroid carcinomas. Nonetheless, it should be possible with a large patient cohort with long-term follow-up to define a set of genes whose expression accurately and independently identifies those thyroid tumors likely to recur locally or metastasize distanty.

Of the few microarray published studies on adrenocortical carcinomas, only one included enough tumors samples to power an outcome analysis. The study by de Fraipont et al. [23] analyzed 57 tumors (33 adenomas and 24 carcinomas) using a limited microarray containing 230 selected genes. Using the resulting expression data, a set of 14 genes were identified that correlated with tumor recurrence, an intriguing result that needs to be validated. A similar unpublished study from my lab identified subgroups of carcinoma with different survival rates. Thus, it appears that microarray analysis will provide an objective way to stratify adrenocortical carcinoma into prognostically-relevant subgroups.

4. Prediction of response to therapy

Admittedly, little work has been done in the area of predicting response to therapy of endocrine tumors. However, based on significant advances in other solid tumor types [35–44], this area holds great promise for endocrine tumors. One of the current limitations of this approach for patient with endocrine tumors is the lack of highly effective therapies for aggressive tumors, such as adrenocortical carcinoma and anaplastic thyroid carcinoma.

One important issue, that if successfully addressed would greatly benefit patients with adrenocortical carcinoma, is the accurate identification of those patients that will benefit from mitotane therapy. The efficacy of mitotane, an adrenolytic agent with a narrow therapeutic window, has been debated for many years due to small study cohorts given the rare nature of the disease. However, a recent retrospective analysis of large Italian and German cohorts showed that mitotane prolonged recurrence-free survival [45]. Since mitotane toxicity is a significant clinical concern that prevents treatment in some patients [46], identifying those patients most likely to benefit is now a priority. A similar argument can be made for predicting response to radioactive iodine therapy for follicular cell thyroid carcinomas, although this therapy is better tolerated than mitotane. It is anticipated that microarray analysis could define sets of genes with predictive power and lead to more discriminative use of both of these therapies.

5. Identification of hormone production via microarray analysis

It may evolve that one of the clinically useful aspects of performing microarray analysis on endocrine tumors will be the comprehensive assessment of hormonal gene expression. This may even be cost-effective. Currently, the standard evaluation of nonfunctioning pancreatic endocrine tumors is to immunohistochemically stain the tumor for the battery of hormones typically made by these tumors in an attempt to identify a tumor marker that can be used to monitor the patient for recurrence. This cost of running this battery of stains is probably not much less than the cost of DNA microarray analysis. Unpublished data from my lab suggests that this is readily and robustly done, as those pancreatic endocrine neoplasms clinically identified as insulinomas have significantly higher levels of insulin transcripts than other tumors. This type of comprehensive hormonal profiling should be true for all types of endocrine tumors and may be cost-effective compared to standard immunohistochemistry when information on numerous hormone levels is desired.

6. Obstacles to clinical implementation of microarray analysis

Transcriptome analysis using commercially available microarrays is costly, whereas analysis using spotted cDNA microarrays is more economical, yet generally considered to be unsuited for clinical testing due to reliability and reproducibility issues. The high cost of microarray analysis will be justified if the results provide vital information not available by other methods such as standard pathologic evaluation. Such information includes selection of an optimal course of therapy, in which case microarray analysis has the potential to save significant health care resources. Thus far, this standard has not been fulfilled for any endocrine tumor. Microarray analysis provides the finest data when the starting mRNA is of high quality, and high quality RNA is most often obtained from fresh-frozen tissues that have been promptly
processed following surgical resection. Commercially-available kits to performed whole genome amplification of RNA have become available recently and, therefore, using formalin-fixed paraffin-embedded tissues as a source of RNA for microarray analysis may be practical in the near future. However, for the time being, fresh-frozen tissue is optimal. Currently, most studies contain samples that either fail to yield quality RNA or fail at microarray analysis. In an unpublished study from my lab on adrenocortical tumors, 87 tissue samples were needed to obtain data on 65 samples. Furthermore, the 87 attempted samples were obtained from over 100 surgeries. Thus, the current overall success rate of microarray analysis for adrenocortical tumors at the University of Michigan is only about 60%. Clearly, this rate is too low for successful clinical implementation. A serious attempt at implementation will require significant revision of surgical and pathology tissue handling procedures to increase the yield of high-quality mRNA and reduce artifact from ischemia, a known cause of gene expression variability [47,48]. Even with improved handling and collection procedures, some percentage of high-grade tumors will yield low-quality mRNA due to extensive in situ tumor ischemia and necrosis.

7. Conclusion

Based on existing studies, the application of DNA microarray analysis to the pathologic evaluation of endocrine tumors holds great promise to improve the care of these patients through improved tumor classification and prognostication, prediction of response to therapy, and assessment of hormone production. Additional studies are needed to further define and validate the utility of microarray analysis and justify the changes in surgical and pathological tissue handling that will be needed to yield technical success in the large majority of cases.

References


