Technical Advances in Rhinologic Basic Science Research

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KEYWORDS

• Chronic rhinosinusitis • Flow cytometry • Real-time PCR
• Sinonasal epithelial cells • Microarray • Polymorphisms
• Animal models

Chronic rhinosinusitis (CRS) is the single most common self-reported chronic health condition in the United States and is estimated to affect 16% of the adult population annually.[1] Despite the prevalence of this disease, there still exists an incomplete understanding of CRS pathophysiology. Due to the lack of effective therapies directed at CRS refractory to medical and surgical therapy, it is critical that we develop a more mature molecular understanding of CRS and CRS with nasal polyps. Research advances over the years have shifted from CRS emerging from sinus ostial obstruction and persistent bacterial infection to newer mechanisms focusing on inadequate host immune responses, persistent bacterial biofilms, and fungal colonization. Technological advances have helped to embrace many of these new mechanisms. In this review, the authors highlight technological advances in rhinology: real-time polymerase chain reaction, epithelial cell culture, flow cytometry, genomics/single-nucleotide polymorphism detection, microarrays, and genetic/nongenetic animal models of sinusitis. The purpose of this review is to describe these methodologies and their contributions toward achieving a better understanding of CRS.

REAL-TIME PCR

The polymerase chain reaction (PCR) has revolutionized molecular research over the past several decades. PCR is a method that allows exponential amplification of short DNA sequences within a longer double-stranded DNA (dsDNA) and requires the use of primers that are complementary to a defined sequence on each of the 2 strands of DNA. These primers are then extended by a heat stable DNA polymerase (Taq

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polymerase) to complete the sequence, leading to logarithmic amplification. Historically, PCR products were run on an agarose gel and stained with an ethidium bromide stain because the reaction was qualitative, determining the presence or absence of a product. Another variation of this technique is reverse transcriptase PCR (RT-PCR), which uses the enzyme reverse transcriptase to convert mRNA to cDNA for use in a PCR reaction. Real-time PCR was developed in efforts to quantitatively assess DNA copy number.[2]

Real-Time PCR: the Technique

Real-time PCR has the ability to monitor the progress of PCR as it occurs ''in real time.'' In this technique, reactions are characterized by the point in time during cycling when amplification of a target is first detected, rather than the amount of target accumulated after a fixed number of cycles. The higher the starting copy number of the gene or nucleotide sequence, the sooner a significant increase in fluorescence is detected. In contrast, conventional PCR and RT-PCR measure the amount of accumulated PCR product at the end of the cycle.

Two primary fluorescence-based sequence detection systems are used: Taqman and SYBR Green chemistry. Taqman chemistry uses fluorogenic labeled probes to enable the detection of PCR product as it accumulates. In the Taqman system, specific hybridization between the probe and target is required to generate a fluorescent signal. Probes can also be labeled with different distinguishable reporter dyes, which can allow for amplification of 2 distinct sequences in 1 tube. The primary disadvantage of this system is that specific probes need to be designed for each reaction. SYBR Green dye is a highly specific dye that binds to all dsDNA. As amplicons are created during PCR, SYBR Green binds to all dsDNA causing an increase in fluorescence proportional to the amount of PCR product created (**Fig. 1**). The primary advantage of the SYBR Green system is that no probe is required, which reduces assay time. This system, however, may generate false-positive signals as SYBR Green dye will bind to any dsDNA.

Real-time PCR data are acquired by a computer and can be analyzed in various ways. Cycle thresholds (C_T) reflect the fractional cycle number of a gene or sequence at which the fluorescence passes the fixed threshold, which is a sample that does not contain a template. The ΔC_T method uses the difference in C_T value obtained between a normalizing housekeeping gene (18 S ribosomal RNA, GAPDH) and the target gene to calculate relative quantification (ΔC_T = the difference in threshold cycles for target and housekeeping gene).[3]

Real-Time PCR Applications in Rhinology

Although conventional PCR and RT-PCR have been used for decades in rhinologic research to evaluate for the qualitative presence of markers/genes in nasal tissue, quantitative real-time PCR has largely grown to replace this technique over the past 5 years. Claeys and colleagues[4,5] and Lane and colleagues initially demonstrated the expression of toll-like receptors (TLRs), human beta defensins, and costimulatory molecules from nasal epithelial cells using real-time PCR. Richer and colleagues[6] demonstrated marked reductions in the level of expression of several genes involved in epithelial barrier maintenance and repair in the inflammatory state of CRS. Furthermore, numerous studies have shown altered sinonasal innate immune epithelial gene expression including TLRs, interleukin 22 (IL22), TLRs, and lactoferrin in patients with CRS compared with normal patients.[7–9] Although this literature review is incomplete, it serves to demonstrate that real-time PCR is a powerful rapid method to screen for alterations in genes/markers between control populations and CRS.