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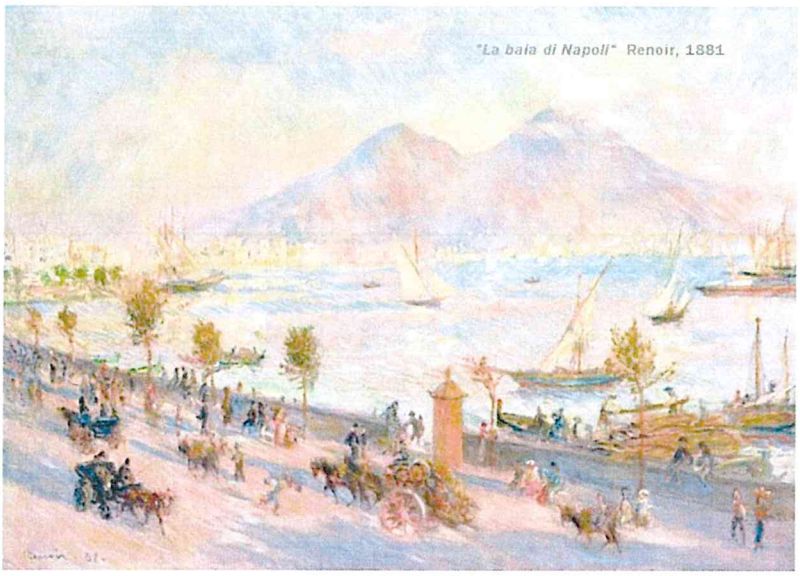

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GENE EXPRESSION STUDY IN A WIDELY USED CELL LINE: MDCK

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Madin-Darby Canine Kidney (MDCK) are cell lines widely used as a models to studying the characteristics of epithelial cells (1), viral infection of cells and in vaccine production (2); however, little is known about MDCK gene expression of parameters involved in the innate immunity response, in DNA repairs, in cell cycle regulation, in the ability to secrete physiologically cytokines, or in response to infectious or non-infectious stressor. Owing to these gaps, that make it difficult to develop standard protocols to produce a vaccine or to study the host pathogen interaction, the aim of our study was to evaluate the basal level of protein release and gene expression of pivotal molecules in the innate immune response and cell cycle regulation. To these purpose, we developed a RT-Real Time PCR to detect the expression of the genes of interest, a selected set of 41 immune-related and epithelial gene transcripts: the immune-related group were TNFA, iNOS, STAT5, IFNG, IL1B, IL2, IL4, IL5, IL6, IL8, IL10, IL12, IL15, IL16, IL17, IL18, IL23, IL27, MYD88, NFK/p65, TLR1, TLR2, TLR3, TLR4, TLR5, TLR7, TLR8, TLR9, TLR10, MD2 and CD14, and the epithelial group were CD44, CXCR4, RAD51, p53, HPRT1, PTEN, Erb2, B2M, GAPDH, B-ACT. MDCK cells were grown to confluence, then washed with PBS and lysed using RTL reagent. Cells were tested at 33rd, 34th and 40th passages; each experiment was repeated ten times. Total RNA extraction, retro-transcription and set-up of RT-PCR reactions were done as previously described (3). GADPH was used as housekeeping gene; RT-PCR analyses were performed in a CFX96 Real-time System. All genes under study were expressed with the exception of IL4, IL10, IL15, IL17, IL27 and IFNG. In particular, IL1B was expressed in 16 out of 18 samples with a DCt of 19.4±1.5, IL2 in 16 of 18 wells (DCt of 20.0±1.2), IL12 in 14 of 18 samples (DCt of 21.2±0.7), while TNFA was unexpressed in 4 of 18 wells (DCt of 20.6±1.2). Regarding TLRs, we obtained: TLR2 expressed in 17 of 18 samples (DCt of 19.4±1.5); TLR4 in 16 of 18 wells (DCt of 18.4±0.6); TLR7 in 12 of 18 samples (DCt of 20.8±2.1); TLR8 in 6 of 18 wells (DCt 21.0±2.3); TLR-9 in 17 of 18 samples (DCt of 20.8±1.3); TLR10 in 14 of 18 analysed wells (DCt 20.6±1.8). The others genes under study were expressed in all samples. Our results, outline the expression of TLR4, MD2, CD14 and TLR5, suggesting the sensibility of MDCK to invasion and penetration of bacterial strain. Moreover, the expression of others TLRs (TLR3, TLR7, TLR8 and TLR9) explains the sensitivity of this cell line to viral infection. In conclusion, our study demonstrate the constitutive expression in MDCK of genes involved in the innate immunity response and cell cycle regulation; then provide the basis to develop usage standard protocols.

[1] Dukes et al., The MDCK variety pack: choosing the right strain, BMC Cell Biology, 12:43, 2011. [2] Gregersen et al., Safety of MDCK cell culture-based influenza vaccines, Future Microbiol, 6: 143-152, 2011. [3] Razzuoli E. et al., IPEC-J2 cells as reporter system of the anti-inflammatory control actions of interferon-alpha. J Interferon Cytokine Res, 33:597–605, 2016.