Molecular pathology of growth anomalies in *Montipora capitata*

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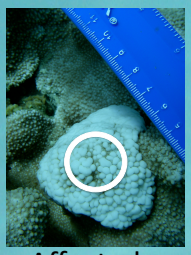
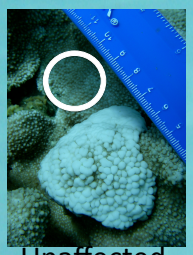
Abstract: Growth anomaly (GA) is a coral disease characterized by enlarged skeletal legions. Although negative effects of GA on several of coral's biological functions have been determined, the etiology and molecular pathology of this disease is very poorly understood. We studied the expression of five genes suspected to play a role in pathological development of GA in the endemic Hawaiian coral, *Montipora capitata,* which is particularly susceptible to this disease. Transcript abundance of the five target genes in healthy tissue, GA affected tissue, and unaffected tissue (tissue adjacent to GA) relative to three internal control genes (actin, NADH, and rpS3) were compared using quantitative reverse transcriptase PCR. Galaxin, which codes for a protein suspected to be involved in calcification and thus hypothesized to be up-regulated in GA, was up- regulated in unaffected tissue, but remained constant in GA tissue. The gene expressions of murine double minute 2 (MDM2) and tumor necrosis factor (TNF) remained constant in GA tissue. The expression of tyrosine protein kinase (TPK) and βγ-crystallin (BGC) were both down-regulated. These expression patterns were all inconsistent with the patterns in neoplastic diseases of similar macromorphology in humans. These expression data therefore suggest that coral GA is not a neoplasia.



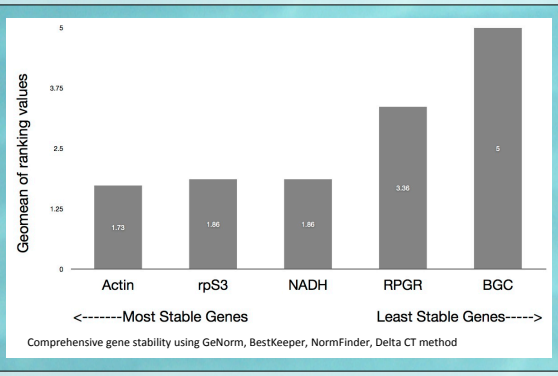
Affected Tissue



Unaffected Tissue

Healthy Tissue

Background: Coral diseases have increased in both severity and incidence in recent years, and have become a serious threat to the coral reef ecosystem. Among the most prominent diseases found in Hawaii, is growth anomaly. Growth Anomaly (GA) is a disease that affects more than 40 species of scleractinian corals in the Caribbean and Indo-Pacific Oceans [1]. GAs are often referred to in the literature as "tumors" or "neoplasms" without a detailed pathological examination to determine that the legions qualify as such [2,3]. True neoplasms are abnormal cells that proliferate and sustain continued growth after an initial stimulus, but does not seem to be the case with the majority of GA cases [1,4,5]. The observance of hypertrophied polyps, and the retention of pigment in the abnormal growths of some coral species has lead to the use of the term "hyperplasia" in contrast to the earlier use of the word "neoplasia" [6]. Hyperplasia is a proliferation in cells, which remain subject to normal cellular regulatory mechanisms, in response to a particular stimulus [7]. While physical evidence suggests that the previously named neoplasia are behaving more like hyperplasia, with no molecular assessment performed. This study examines the molecular pathology of GAs in *Montipora capitata* by examining the gene expression of four oncogene homologs. We also examined the expression of skeletal organic matrix protein, galaxin, whose genetic homolog collagen has an altered expression under neoplastic conditions.



Methods: Healthy, unaffected (apparently healthy tissue adjacent to GA) and GA affected *M. capitata* coral samples were collected from Wai'opae, an area with high prevalence of GA, on Hawaii island [8]. Tissue was removed and total RNA was extracted using a TRIzol/RNeasy hybrid extraction method. Three different series of RNA were extracted and each was reverse transcribed to cDNA. qPCR was performed on 4 oncogene homologs (BGC, MDM2, TNF, TPK) and Galaxin, using TaqMan double quenched probes. The assays were run alongside three internal control genes (Actin, NAHD, rpS3) that were shown to be constitutively expressed among our tissue types. Internal control genes were chosen using a comprehensive algorithm, assuring they are constitutiveley expressed among treatments. qPCR was performed using TaqMan Gene Expression Mastermix on an ABI StepOne Plus machine.

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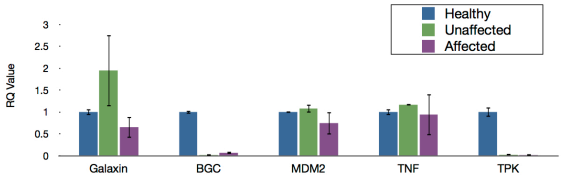

Normalized relative transcript abundance of target genes in healthy, unaffected, and affected tissues of the coral *Montipora capitata*

Results: Our results show that there is no difference in transcript abundance in MDM2 and TNF genes. There was a significant difference among expression of BGC (ANOVA, f= 83.85 ,p<0.001, Tukey's HSD) and TPK (ANOVA, f= 48.61, p<0.001, Tukey's HSD). The transcript abundance of galaxin showed no differences between healthy and affected nor between healthy and unaffected, but significantly higher between unaffected than affected tissue (ANOVA, f= 4.93, p=0.016, Tukey's HSD).

	Gene	Function	Expected in neoplasia	Actual
	Galaxin	Structural organic matrix support, possibly calcification	↑	=
Oncogenes	BGC	Differentitation, tumorigenicity, cell morphology	↑	↓
Oncogenes	MDM2	Regulates a tumor suppressor gene	↑	=
Oncogenes	TNF	Immune response, apoptosis, necrosis	↑	=
Oncogenes	TPK	Differentiation, apoptosis, immune response	↑	↓

Discussion: The expression patterns of oncogene homologs in *Montipora capitata* GA in this study were inconsistent with those expected for neoplasia. TNF and MDM2 expression remained constant among tissue types. The expression of TNF is upregulated in a wide variety of human cancers [9], however it was unchanged in both GA-affected and unaffected tissues, when compared to healthy corals. The expression of MDM2, which regulates the tumor suppressor gene p53, is often increased in the presence of neoplasia and we observed no change in expression [10]. Similarly, TPK is often over-expressed under neoplastic conditions in humans [11]. In contrast, TPK and BGC both showed a decrease in expression level in both unaffected and affected tissue types compared to healthy, in *M. capitata*. BCG has also been shown to be overexpressed in several cancer types [12]. The results of BCG expression were not consistent with those expected if GAs are a neoplastic condition. Our results show that the expression of galaxin remains relatively unchanged in GA-affected tissue. However, galaxin expression in unaffected tissue showed nearly a two-fold increase in expression. It is possible that this increase in galaxin expression is a result of metabolic activities within the GA. Galaxin is homologous to collagen, which is typically up-regulated in human neoplasia. The expression of collagen has been shown to be up-regulated in tissues associated with tissue repair in response to pathogens that cause inflammation [13]. It is possible that the tissues adjacent to GA are responding to the diseased tissue and attempting to repair it. Some scleractinians are capable of responding to an invasion of microbes by inducing calicoblasts to lay down a barrier composed of layers of skeleton and organic material to prevent further contamination [14]. The increase in galaxin expression may also indicate that the unaffected tissue is attempting to lay down such a barrier in between itself and the GA to avoid infection, if this disease is indeed pathogenic.

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