gli-1 Oncogene Is Highly Expressed in Granulomatous Skin Disorders, Including Sarcoidosis, Granuloma Annulare, and Necrobiosis Lipoidica Diabeticorum

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Background: Sarcoidosis, which occurs most commonly in African American women, is a granulomatous multisystem disorder affecting the skin, lungs, and central nervous system. In a previous immunohistochemistry study of keloids, a scar granuloma stained highly positive for glioma-associated oncogene homologue (gli-1).

Observation: This observation led us to study whether gli-1, one of the vertebrate zinc finger transcription factor genes of the gli superfamily, is expressed in granulomatous skin disorders such as cutaneous sarcoidosis, granuloma annulare (GA), and necrobiosis lipoidica diabeticorum (NLD). Immunohistochemistry studies for gli-1 were performed on biopsy specimens from patients with cutaneous sarcoidosis, GA, and NLD. All sarcoid lesions were highly positive for gli-1 expression, and 75% of the cells demonstrated positivity with a stain intensity of 3 on a scale of 1 to 3. The gli-1 expression was confined to cutaneous granulomas. CD68 staining was highly positive in the sarcoid lesions as well. Similarly, GA and NLD lesions were uniformly positive for gli-1 expression.

Conclusions: We found that gli-1 is inappropriately expressed in granulomatous lesions of the skin such as cutaneous sarcoidosis, GA, and NLD. These findings provide a rationale for clinical trials of inhibitors of gli-1 signaling, including tacrolimus and sizolimus, for the treatment of cutaneous sarcoidosis and other granulomatous disorders of the skin.

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GRANULOMATOUS DISORDERS of the skin include cutaneous sarcoidosis, granuloma annulare, (GA), and necrobiosis lipoidica diabeticorum (NLD). The etiology of these disorders is unknown. Sarcoidosis has been associated with chronic inflammation and has recently been described in patients receiving interferon α and ribavirin, implying that sarcoidosis may be a T-cell-type of inflammatory reaction. Histologically the granulomas may simulate mycobacterial granulomas, but tissue cultures have not evidenced organisms. Sarcoidosis is a common disorder with significant morbidity and mortality, especially pulmonary sarcoidosis. It can simultaneously affect sites as varied as the skin, kidney, heart, and central nervous system, where it may cause diabetes insipidus. Treatment of sarcoidosis usually involves long-term systemic treatment with corticosteroids and other potent immunosuppressive agents, with resulting adverse effects. When sarcoidosis affects only the skin it poses a therapeutic dilemma. On the one hand, lesions respond much better to systemic than to topical steroids; on the other, when sarcoidosis is limited to the skin, systemic steroids may cause disproportionate unwanted effects. Other granulomatous disorders such as GA and NLD appear to involve primarily the skin, but they may occur elsewhere in patients with systemic disorders such as diabetes mellitus, hyperlipidemia, and multiple myeloma. Again, treatment of these disorders is unsatisfactory. Granuloma annulare may resolve spontaneously after several years but NLD is less likely to resolve spontaneously.

In an earlier study, after an anecdotal report that keloids responded to topical tacrolimus, our group reported that keloids expressed high levels of both gli-1 (glioma-associated oncogene homo-
logue) oncogene and inhibitor of gli-1 signaling. In that study, we noted strong gli-1 expression in a suture-induced granuloma, and hypothesized that noninfectious granulomas may express gli-1 protein and that these granulomas may be treated with inhibitors of gli-1 signaling.

**METHODS**

Paraffin blocks of biopsy samples of 10 cutaneous sarcoidosis, 3 GA, and 5 NLD lesions were stained with antibodies against gli-1 oncogene, as previously performed on keloids. Briefly, 3-µm sections of formalin-fixed, paraffin-embedded tissue were tested for the presence of gli-1 using gli-1-specific antibodies (Santa Cruz Biotechnology, Santa Cruz, Calif) with an avidin-biotin complex technique and steam heat–induced antigen retrieval in a 1:20 ratio. An avidin-biotinylated enzyme complex kit (Dako LSAB2, Dako Corp, Carpinteria, Calif) was used in combination with the automated Dako Autostainer. Hematoxylin was used as a counterstain. Sarcoid lesions were stained with CD68 as well. The staining was done using an avidin-biotin–complex technique with pressure cooker heat–induced antigen retrieval and a Dako Autostainer. The percentage and intensity of positivity for gli-1 and CD68 were microscopically quantitated and recorded. The intensity of positivity was recorded on a 3-tiered scale graduated from 0 to 3 and the intensity of staining was recorded on a 4-tiered scale as follows: 0% to 5%, 0; 6% to 25%, 1; 26% to 50%, 2; 51% to 75%, 3; and 76% to 100%, 4. Statistical analyses were performed with the statistical package SPSS (SPSS Inc, Chicago, Ill).

**RESULTS**

All cutaneous sarcoid lesions showed high-level gli-1 expression in nearly all cells (Figure 1). Staining was predominantly cytoplasmic, similar to what has been observed in basal cell carcinoma. Cutaneous sarcoids also demonstrated strong expression for CD68, which is consistent with the known strong expression of CD68 in this disorder (Figure 2). Strong gli-1 expression was also observed in lesions of GA (Figure 3) and NLD (Figure 4), ie, to areas of granulomas. There was no difference in intensity of staining between the 3 granulomatous disorders (Table). While there were differences between the 3 disorders in the percentage of cells that stained for gli-1, the difference was not significant (P = .24 by analysis of variance).

**COMMENT**

An oncogene that was initially characterized as a gene amplified in a human glioma, gli-1, hence the name gli-1, has been shown to transform cells in culture, and, more recently, to be involved in the pathogenesis of basal cell
A transcriptional activator, gli-1, is downregulated by the PATCHED (PTC) gene, which is a receptor for the protein sonic hedgehog (Shh). The PTC gene represses gli-1 transcription, and when PTC is absent, as in basal cell nevus syndrome or sporadic basal cell carcinoma, gli-1 transcription is stimulated. Indeed, transgenic overexpression of gli-1 in mice leads to diffuse development of basal cell carcinoma.

We have previously found that gli-1 is highly expressed in keloids but not in hypertrophic scars. A suture-induced granuloma also stained positively for gli-1, suggesting that granulomas may also express gli-1. Mesenchymal cells in keloids have been shown to express CD68, a marker also commonly observed in granulomatosus skin disease, suggesting that gli-1 may be expressed in granulomas as well as keloids. To determine whether this was the case, we stained specimens of cutaneous sarcoidosis, GA, and NLD. All specimens of granulomatosus skin disorders stained strongly positive for gli-1. These results demonstrate a rationale for the use of inhibitors of gli-1 signaling, such as topical tacrolimus, in the treatment of granulomatosus skin disorders. Indeed, a case report of a patient responding to tacrolimus (Protopic) for cutaneous sarcoidosis has been described.

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