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p120-Catenin Is Required for Dietary Calcium Suppression of Oral Carcinogenesis in Mice

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Abstract

Previous studies have shown that dietary calcium suppresses oral carcinogenesis, but the mechanism is unclear. p120-catenin (p120) is a cytoplasmic protein closely associated with E-cadherin to form the E-cadherin–β-catenin complex and may function as a tumor suppressor in the oral epithelium. To determine whether p120 is involved in the mechanism by which dietary calcium suppresses oral carcinogenesis, The normal, low, or high calcium diet was fed control mice (designated as floxed p120 mice) or mice in which p120 was specifically deleted in the oral squamous epithelium during the adult stage (designated as p120cKO mice). All mice were exposed to a low dose of oral cancer carcinogen 4-nitroquinoline 1-oxide and rates of oral squamous cell carcinoma (OSCC) and proliferation and differentiation in the cancerous and non-cancerous oral epithelium of these mice were examined. The results showed that the low calcium diet increased rates of OSCC and proliferation of the non-cancerous oral epithelium and decreased differentiation of the non-cancerous oral epithelium, but had no effect on cancerous oral epithelium. In contrast, the high calcium diet had opposite effects. However, the effect of the dietary calcium on the rates of OSCC, proliferation, and differentiation of the non-cancerous epithelium were not seen in p120cKO mice. Based on these results, we conclude that p120 is required for dietary calcium suppression of oral carcinogenesis and oral epithelial proliferation and dietary calcium induction of oral epithelial differentiation.

Numerous studies have indicated that a high intake of calcium and vitamin D are associated with a reduced risk of some types of cancers (Garland et al., 1985; Kampman et al., 2000; Shin et al., 2002; Wu et al., 2002; McCullough et al., 2005). In a 4-year trial,

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Lappe et al. (2007) reported that a non-significant 47% reduction in total cancer risk with calcium monotherapy, and significant 60% reduction with calcium plus vitamin D in postmenopausal women. They conclude that improving calcium and vitamin D nutritional status substantially reduces all-cancer risk in postmenopausal women. We have recently shown that high calcium intake suppresses oral carcinogenesis and low calcium intake promotes oral carcinogenesis in mice (Jiang et al., 2015), suggesting that calcium has protective effects on oral carcinogenesis. However, the mechanism by which dietary calcium exerts this effect is unclear.

Extracellular calcium is potent inducer of keratinocyte differentiation (Hennings et al., 1989; Menon et al., 1992; Bikle et al., 2001). p120-catenin (p120) is a cadherin-associated protein that stabilizes cadherin and is ubiquitously expressed in epithelial tissues. p120-dependent E-cadherin–β-catenin–p120-catenin complex in the plasma membrane triggers an intracellular signaling pathway essential for extracellular calcium-induced keratinocyte differentiation (Xie and Bikle, 1999, 2007; Xie et al., 2005, 2009). Reduced p120 expression has been frequently observed in a number of human epithelial cancers and correlates with tumor progression and poor prognosis (Kallakury et al., 2001; Ishizaki et al., 2004; Wang et al., 2006; Chung et al., 2007; Liu et al., 2007; Zhai et al., 2008). Deletion of p120 in the oral keratinocytes beginning at the embryonic stage through adulthood of the mouse results in loss of E-cadherin and activation of decreased keratinocyte differentiation and increased keratinocyte proliferation in the oral epithelium, development of an inflammatory microenvironment, and spontaneous OSCC (Stairs et al., 2011). These observations suggest that p120 plays a critical role in stimulating keratinocyte differentiation and inhibiting keratinocyte proliferation in the oral epithelium as central to its ability to suppress oral carcinogenesis.

Given that p120 plays a critical role in regulating differentiation and proliferation of oral keratinocytes and oral carcinogenesis, we hypothesized that p120 is required for dietary calcium-regulated keratinocyte proliferation and differentiation and oral carcinogenesis in the oral epithelium. To test this hypothesis, we examined OSCC formation, oral keratinocyte differentiation, and proliferation in p120 conditional knockout mice (p120cKO) fed diets containing different calcium contents.

Materials and Methods

Generation of p120cKO mouse models and 4NQO-induced oral carcinogenesis model

In this study, we used C57/BL6 mice in which the p120 gene was deleted specifically in keratinocytes and OSCC cells in their squamous epithelium including oral epithelium, with the deletion being initiated by tamoxifen administration. Briefly, these mice (designated as floxed p120/Cre mice) were initially produced by breeding mice with floxed exons 3–8 of p120 (designated floxed p120 mice, a gift from Dr. Albert Reynolds) (Davis and Reynolds, 2006) to K14-Cre-ER^{T2} mice (designated K14-Cre mice, a gift from Dr. Pierre Chambon) expressing tamoxifen-regulated Cre-recombinase (Cre) targeted to keratinocytes and OSCC cells in the squamous epithelium using the keratin 14 (K14) promoter. Because Cre recombinase in K14-Cre mice is under the control of the keratin 14 promoter that is specifically active in the squamous epithelial basal layer and the activity of Cre

recombinase is induced by tamoxifen, the floxed p120 gene segment was excised through Cre recombinase-mediated recombination only in the squamous epithelia basal layer of tamoxifen-induced floxed p120/Cre mice. The floxed p120 mouse has also been used previously to target p120 ablation to the salivary skin (Perez-Moreno et al., 2006). Floxed p120 mice were used as controls. One hundred and twenty floxed p120/Cre mice were randomly divided into the high, normal, and low calcium diet groups with 40 in each group. One hundred and twenty floxed p120 mice were used as controls, which were randomly divided into the high, normal, and low calcium diet groups with 40 in each group. Tamoxifen was injected into the abdominal cavity of each mouse three times in a week (1 mg/time) to induce deletion of p120 from floxed p120/cre mice at 4 weeks of age. These mice lack p120 specifically in keratinocytes and are designated as p120cKO mice. Each group of mice was fed a normal chow diet (Envigo Teklad Diets, Madison, WI, #8664, 1.3% calcium, 1.0% phosphorus, 2200 IU vitamin D/kg diet), a high calcium chow diet (Envigo Teklad Diets, Madison, WI, Cat# 96348, 2.0% calcium, 1.25% phosphorus, 2200 IU vitamin D/kg diet), or a low calcium chow diet (Envigo Teklad Diets, Madison, WI, Cat# 92027,0.01 % calcium, 0.4% phosphate, 2200 IU vitamin D/kg diet) at 4 weeks of age. All mice were also fed drinking water containing 4-nitroquinoline 1-oxide (4NQO, 10 μg/ml, Sigma–Aldrich, St. Louis, MO) for 16 weeks to induce oral carcinogenesis. 4NQO is a chemical carcinogen is known to selectively induce oral carcinogenesis (Nakahara et al., 1957; Steidler and Reade, 1986; Hawkins et al., 1994; Yuan et al., 1994). The mice were then fed normal drinking water for 12 weeks. At the end of 12 weeks, these mice were sacrificed and their tongues were removed for the subsequent analysis. Figure 1 shows the timeline of the tamoxifen, 4NQO, and calcium treatments. All of these mice were maintained under specific-pathogen-free conditions. Animals were housed under a 12 h light–dark cycle in a temperature- and humidity-controlled room and received ad libitum access to food and water in the animal facility of the San Francisco VA Medical Center. The study was approved by the Animal Care and Use Committees in the San Francisco VA Medical Center according to the guidelines of the Animal Welfare Act and all applicable state and federal laws.

OSCC rates and histological assessment

Tumors on each tongue were visually counted. The number of tumors was recorded. To determine the rates of OSCC, a complete histopathological examination of the tongue from each mouse was performed. After checking the overt tumor phenotypes, the entire tongue of each mouse were embedded in paraffin and sectioned, and histopathological analysis with haematoxylin and eosin (H&E) staining was performed on every 20th section of each tissue. The entire epithelium on each section was examined for the presence of tumors microscopically. The tumor was then examined for the presence of atypia and invasion. The number of tumors including papillomas and OSCC was counted microscopically. The proportion of papillomas to OSCC per animal was calculated. The results were reviewed by two pathologists at the Second Xiangya Hospital, Central South University in China. Selected sections were examined by immunohistochemical analysis. For immunohistochemical analysis, paraffin-embedded 4-mm-thick specimens were dewaxed in turpentine and rehydrated through decreased concentrations of ethanol. Antigen retrieval was not performed. Endogenous peroxidase activity was blocked by using 3% H_2O_2 in methanol for 15 min. The sections were incubated with trisodium

citrate dihydrate solution (0.125%, pH 6.0) for 15 min, and then soaked with phosphate buffered saline (PBS) liquid (pH 7.2–7.4) three times for 5 min. The sections were then pre-incubated with sheep serum for 10 min to block non-specific antigen. The pretreated slides were incubated overnight at 4°C in a humidified chamber with rabbit polyclonal or monoclonal primary antibodies against mouse p120, PCNA, keratin 1, involucrin, loricrin, and filaggrin. Antibodies purchased from Santa Cruz Biotechnology (Santa Cruz, CA) include rabbit polyclonal antibodies against proliferating cell nuclear antigen (PCNA, cat# sc-7907) and p120 (cat# sc-1101). Antibodies purchased from Covance Research Products (Denver, PA) include rabbit polyclonal antibodies against keratin 1 (cat# PRB-1499-100, dilution 1:500), involucrin (cat# PRB-140c, dilution 1: 1500), loricrin (cat# PRB-145p-100, dilution 1: 1000), or filaggrin (cat# PRB-417p-100, dilution 1:3000). The slides were then incubated at room temperature for 1 h, rinsed with PBS three times, incubated with appropriate biotinylated secondary antibodies for 20 min followed by avidin (Maixin Biological Technology Development Company) and diaminobenzidine (Maixin Biological Technology Development Company). Hematoxylin was used as counter-staining. In the negative controls, PBS (pH 7.4) was used instead of the primary antibody. For the quantification of immunohistochemistry, all sections were viewed 10 fields per section and analyzed with an image analysis program (Bioquant, Nashville, TN) using the methods, as described previously (Al-Shatti et al., 2005; Fedorczyk et al., 2010). Six sections from each mouse were stained for each protein. All the histological experiments were performed at the Second Xiangya Hospital, Central South University in China.

Quantitative real time PCR

Before assigning to each experimental group, one floxed p120 mouse and one p120cKO mouse were randomly selected from each litter to check p120 protein and mRNA knockdown. Briefly, total RNA from the mucosa separated from the tongue was extracted using RNA-STAT 60 (Tel-Test, Inc., Friendwood, TX). Concentration and purity were determined by measuring the absorbance at OD 260/280 nm. About 1 μg of RNA was reversely transcribed using random hexamers with the TaqMan reverse transcription kit (Applied Biosystems, Foster City, CA). The amount of cDNA was then quantified by quantitative real time PCR, performed on a PE Biosystems model 7900 HT sequence detector at San Francisco VA Medical Center. The PCR amplification was done using SYBRGreen Universal PCR Master Mix (Applied Biosystems) and primers (Forward: 5′-GGCTCGATATGGACCTTAC-3′; Reverse: 5′-ACTCAATATGGCGTGGCTTAC-3′). Levels of mRNA were normalized to mitochondrial ribosomal protein L19.

Determination of serum calcium, phosphate, and intact parathyroid hormone

Concentrations of calcium and phosphate in serum were determined using an automatic biochemical analyzer (Abbott Laboratories, North Chicago, IL). Concentrations of intact parathyroid hormone (iPTH) were measured by enzyme linked immunosorbent assay (ELISA) (ALPCO Diagnostics, Salem, NH) performed according to the manufacturer's instructions.

Statistical analysis

The sample size was calculated so as to give the study a 95% probability of detecting a 35% difference in tumor rates among groups. The Chi-squared test was used to compare tumor rates in floxed p120 or p120cKO mice on the low, normal, and high calcium diet. One-way (2-factor) ANOVA was used to compare levels of mRNA and protein and concentrations of calcium, phosphate, and iPTH in serum from floxed p120 or p120cKO mice fed the low, normal, and high calcium diet. Comparison between the groups was performed using Student–Newman–Keuls (S–N–K) method. *P*-values < 0.05 were considered significantly different among groups. For all analysis, the software SigmaStat version 3.5 (Systat Software Inc., San Jose, CA) was used.

Results

Verification of p120 deletion in p120cKO mice

To verify p120 loss in tamoxifen-induced floxed p120/Cre mice (p120cKO mice), p120 mRNA and protein expression in the squamous cell epithelia were assessed by quantitative PCR and immunohistochemistry, respectively. The results showed that p120 mRNA and protein were lost in the oral squamous epithelium of p120cKO mice compared to floxed p120 mice (Fig. 2).

Deletion of p120 compromised calcium-suppressed oral tumor formation

Although oral cancers most commonly involve the tongue, we examined the tongue of the mouse for appearance, size, and number of the tumors and collected the tongue tissue for further histological analysis. The H&E staining of the tongue samples of these mice revealed that 90–95% of the tongue tumors in each of floxed p120 and p120cKO mice with oral tumors were squamous cell carcinoma (SCC) and 5–10% of those were papillomas. There was no significant difference in the number of tumor counted visually or microscopically, the size of tumor, and the proportion of papillomas to SCC per animal in each group. Furthermore, the tongue SCC displayed invasion into the basal membrane and interstitial tissue compared to papillomas displayed no invasion. Figure 3 shows the representative sections of the papilloma without invasion and the SCC with tumor invasion. Carcinomas were subclassified from grades I to IV, based on the degree of keratinization within the cancer. Each mouse was scored based on the most severe degree of disease found on any of the sections from its tongue. The data were analyzed by two-factor ANOVA. After demonstrating that the interaction between diet and genotype was significant ($P < 0.05$), we analyzed the data from each diet separately by ANOVA. The results showed that 19 out of 40 mice (47.5%) on the low calcium diet, 8 out of 40 mice (20.0%) on the normal calcium diet, and 2 out of 40 mice (5.0%) on the high calcium diet developed OSCC in the tongue among floxed p120 mice (Fig. 4). In contrast, 19 out of 40 mice (47.5%) on the low calcium diet, 17 out of 40 mice (42.5%) on the normal calcium diet, and 15 out of 40 mice (37.5%) fed the high calcium diet developed OSCC among 120 p120cKO mice (Fig. 4). Statistical analysis showed that the low calcium enhanced rates of OSCC and the high calcium diet significantly reduced rates of OSCC in floxed p120 mice (Fig. 4). However, deletion of p120 in the tongue epithelium abolished effects of calcium on the rates of OSCC (Fig. 4). Under microscope, all carcinomas were shown grade 1 tumors. These data suggest that dietary

calcium suppresses OSCC formation and p120 is required for dietary calcium-suppressed OSCC formation.

Ablation of p120 compromised calcium-suppressed proliferation of normal oral epithelium

To determine whether p120 is required for dietary calcium suppression of proliferation of oral epithelium, we examined the expression levels of PCNA in the oral epithelium of p120cKO and floxed p120 mice fed the three different calcium diets by immunohistochemistry. The results showed that the level of PCNA in the non-cancerous oral epithelium of floxed p120 mice was significantly higher in the low calcium diet group and significantly lower in the high calcium diet group compared to that in the normal calcium diet group (Fig. 5) although there was no significant differences in the levels of PCNA in OSCC among different calcium groups (data not shown). However, the effect of the dietary calcium on the levels of PCNA in the normal oral epithelium was abolished in p120cKO mice (Fig. 5). These data indicate that p120 is required for dietary calcium-suppressed proliferation of normal oral epithelium.

Ablation of p120 compromised dietary calcium-promoted differentiation of normal oral epithelium

To determine whether p120 is required for calcium induction of differentiation of the normal oral epithelium, we examined expression levels of epidermal differentiation markers in the normal oral epithelium in floxed p120 mice and p120cKO mice using immunohistochemistry. The results showed that levels of differentiation markers (keratin 1, involucrin, loricrin, and filaggrin) in the non-cancerous oral epithelium of floxed p120 mice were significantly lower in the low calcium diet group and significantly higher in the high calcium diet group than the normal calcium diet group (Fig. 6) although there was no significant differences in the levels of differentiation markers in OSCC among different calcium groups (data not shown). These data suggest that dietary calcium promotes differentiation of the normal oral epithelium. However, there was no significant difference in the expression levels of differentiation markers in the normal oral epithelium of p120cKO mice among three groups (Fig. 6); rather all levels were equivalent to that in the low calcium diet group. These results indicate that p120 is required for dietary calcium induction of differentiation of the normal oral epithelium.

Dietary calcium and p120 ablation did not alter levels of calcium and phosphorus but raised iPTH levels in the serum of the mice

To determine whether dietary calcium or ablation of p120 affects levels of calcium, phosphorus and iPTH in the circulation, we examined the concentrations of calcium, phosphate, and iPTH in the fasting serum sample of floxed p120 and p120cKO mice fed the low, normal, or high calcium diet. The results showed that there was no significant difference in calcium and phosphate among three groups of mice, but there was a significant increase in the level of iPTH in serum of mice fed the low calcium diet and there was a significant decrease in the level of iPTH in serum of mice fed the high calcium diet, compared to that of mice fed the normal calcium diet (data not shown). However, p120 deletion did not affect PTH levels (Fig. 7).

Discussion

The present study was designed to determine whether p120 is required for dietary calcium-induced oral carcinogenesis. To reach this goal, we generated a conditional knockout model in which p120 was deleted specifically in squamous epithelium and assessed the predisposition of p120cKO mice fed three different calcium diets to chemically induced OSCC. Our results showed that the incidence of OSCC in floxed p120 mice was decreased in the high calcium diet group and increased in the low calcium diet group compared to the normal calcium diet group, suggesting that calcium suppresses oral carcinogenesis. However, no obvious differences in the rate of OSCC in the oral epithelium of p120cKO mice among three groups were observed, remaining equivalent to that of the low calcium diet in the controls, indicating that p120-catenin is required for dietary calcium-suppressed oral carcinogenesis. Our recent studies have also shown that the high calcium diet induces differentiation, and suppresses proliferation and carcinogenesis, in the oral epithelium (Jiang et al., 2015). Our present studies have confirmed this phenomenon and further uncovered the underlying mechanism. The data support our hypothesis that p120, as a tumor suppressor, is required for dietary calcium-suppressed oral carcinogenesis.

Other studies including those by Liu et al. (2007) in lung carcinoma and Mayerle et al. (2003) in pancreatic carcinoma have shown the role of p120 in suppressing proliferation of these cells. Perez-Moreno et al. (2008) have shown that epidermal hyperproliferation occurs in the absence of p120. Our present data have shown that high dietary calcium reduced and the low dietary calcium promoted proliferation in the non-cancerous oral epithelium but not in OSCC, and deletion of p120 in the oral epithelium blocked these effects. These data indicate that dietary calcium inhibits proliferation in the normal oral epithelium but not in OSCC. The data also indicate that p120 is required for calcium-suppressed proliferation in the non-cancerous oral epithelium.

We have previously reported that the signaling pathway involving calcium-induced formation of the E-cadherin–β-catenin–p120 complex in the plasma membrane and a subsequent rise in the intracellular calcium mediates calcium-induced differentiation of epidermal keratinocytes (Xie and Bikle, 1999, 2007; Xie et al., 2005, 2009). Although these studies were primarily performed in epidermal keratinocytes, the findings are possibly applicable to oral keratinocytes, as indicated by studies showing that mice lacking p120 in oral keratinocytes displayed reduced differentiation of oral keratinocytes (Stairs et al., 2011). Our present data showed that the high calcium diet promoted and the low calcium diet reduced the expression of differentiation in the non-cancerous oral epithelium but not in OSCC and knockdown of p120 abolished these effects. These data suggest that p120 is required for dietary calcium induced differentiation of oral keratinocytes.

Although calcium suppresses PTH secretion, we examined iPTH, calcium, and phosphorus levels in floxed p120 mice and p120cKO mice fed different calcium diets. The results showed that the dietary calcium did not alter levels of calcium and phosphorus, but suppressed PTH. It is unclear whether altered PTH regulates oral carcinogenesis, proliferation, and differentiation of oral epithelium. Further studies are needed to answer this question.

Taken together, our results raise new insights in explaining why dietary calcium suppresses oral carcinogenesis and indicate that p120 plays a critical role in dietary calcium-suppressed oral carcinogenesis. In other words, dietary calcium would have no inhibitory effect on oral carcinogenesis if p120 is lost. However, a limitation of the present studies is that findings in murine studies may not be directly extrapolated to the human situation. Additional studies in humans are warranted, giving that high calcium intake is associated with a reduced risk of cancers. Another limitation is that our experimental design could not address how p120 regulates oral carcinogenesis. We hypothesize that the critical role of p120 in stabilizing the E-cadherin/catenin complex in the membrane of keratinocytes, a complex central to calcium regulation of proliferation and differentiation of these cells, is likely the reason that deleting p120 reduces the ability of these cells to respond to calcium. Thus in the absence of a functioning E-cadherin/catenin complex, the proliferation proceeds unchecked and differentiation is inhibited regardless of the extracellular calcium leading to oral carcinogenesis. Recent animal studies have demonstrated that deletion of p120 in the oral epithelium beginning at the embryonic stage though adulthood of the mouse results in loss of E-cadherin and activation of NFkB and STAT3 as well as decreased differentiation and increased proliferation in the normal oral epithelium and OSCC (Stairs et al., 2011). These observations suggest that NFkB and STAT3 may play a role in p120 suppression of OSCC carcinogenesis. Further studies are required to address whether dietary calcium suppresses oral carcinogenesis via inhibiting NFkB and STAT3 signaling pathways.

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Abbreviations:

Cao	extracellular calcium
Cai	intracellular calcium
EGF	epidermal growth factor

EGFR	epidermal growth factor receptor
IP_3	inositol trisphosphate
OSCC	oral squamous cell carcinoma
p120	p120 catenin
PIP5K1α	phosphatidylinositol-4-phosphate 5-kinase 1α
PLC-γ1	phospholipase C-γ1
PIKE	phosphatidylinositol 3-kinase enhancer
PCNA	proliferating cell nuclear antigen
PI3K	phosphatidylinositol-3-kinase
PIP_3	phosphatidylinositol (3,4,5)-triphosphate
p120cKO	p120 conditional knockout
SCC	squamous cell carcinoma
4NQO	4-nitroquinoline 1-oxide

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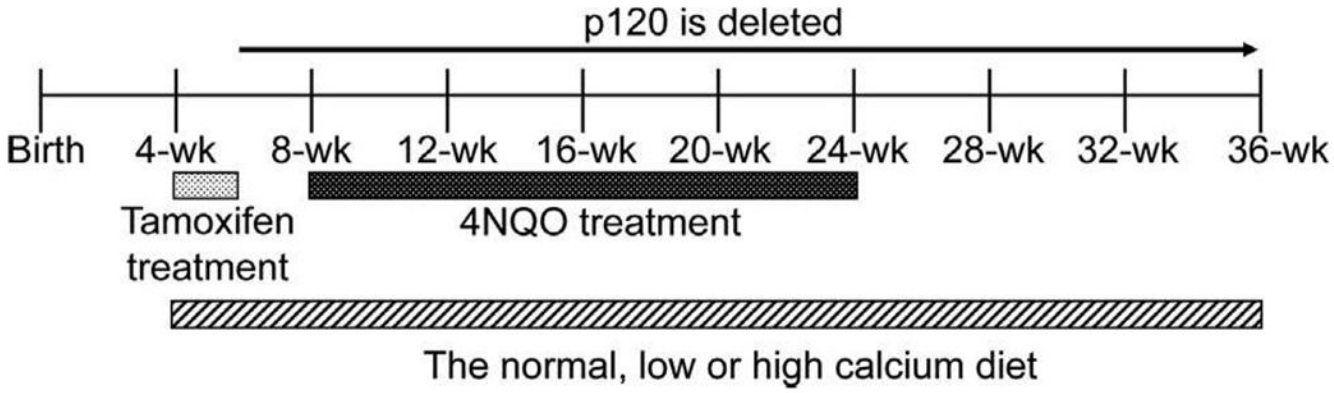

Fig. 1.

The timeline of the tamoxifen, 4NQO and calcium treatment. Mice were treated with tamoxifen followed by 4NQO and calcium as described in Materials and Methods section.

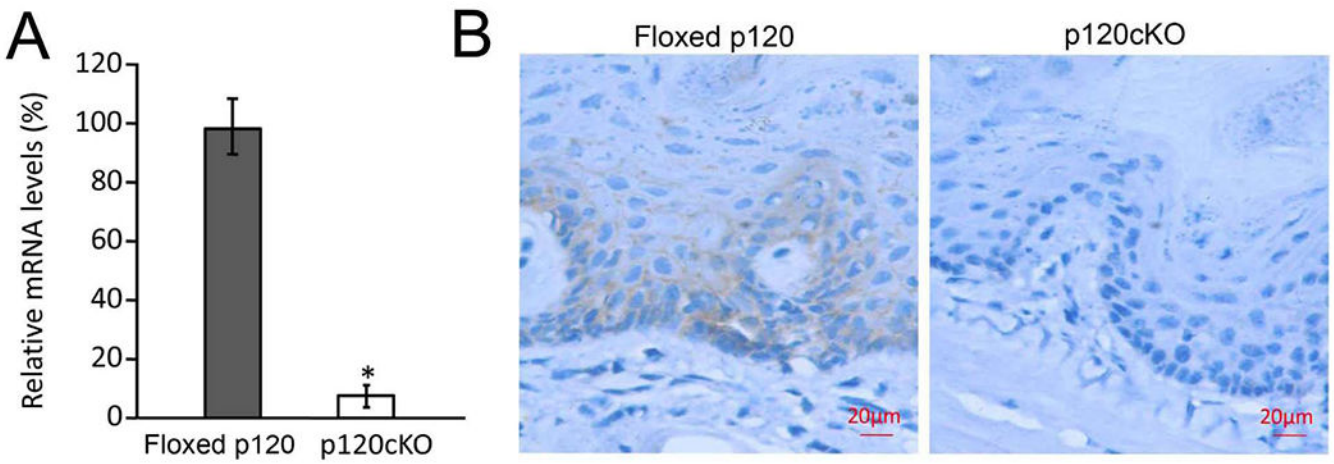

Fig. 2.

Ablation of p120 gene in mouse oral epithelium. (a) Quantitative PCR (qPCR) analysis of RNA isolated from the oral epithelium of one mouse from each litter before feeding different calcium diet and 4NQO. The level of p120 mRNA in p120 epidermis was normalized to that in controls and is presented as mean ± SD, n = 6. The Student's *t*-test was used to determine differences between groups. Statistical significance (*$P < 0.01$) between floxed p120 and p120cKO mice is shown. (b) Immunohistochemical staining of the tongue for p120. The positive staining is shown as brown. The figure shows a representative section of all of 40 mice in each group.

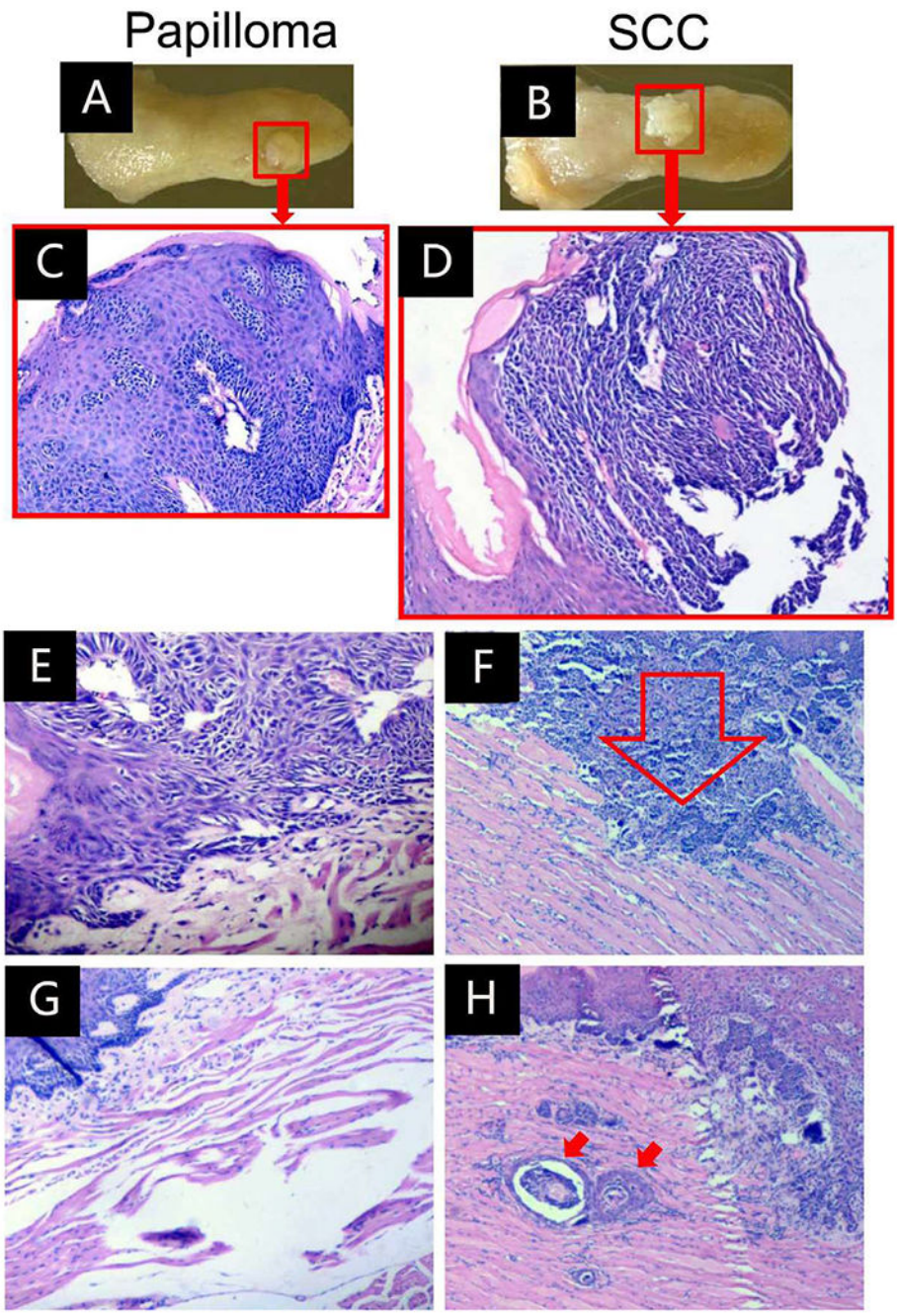

Fig. 3.

Invasion of OSCC. Parts (a and b) show the appearance of a representative papilloma and SCC in the tongue from all of 40 mice in each group. Parts (c–h) show H&E staining of the papilloma and SCC. The open arrow in part (f) indicates the muscular invasion of SCC and the filled arrow in part (h) indicates the SCC invasion into the intrinsic tongue muscle layer. The H&E staining were performed on the sections of all papillomas and SCC. The figure shows staining of a representative section.

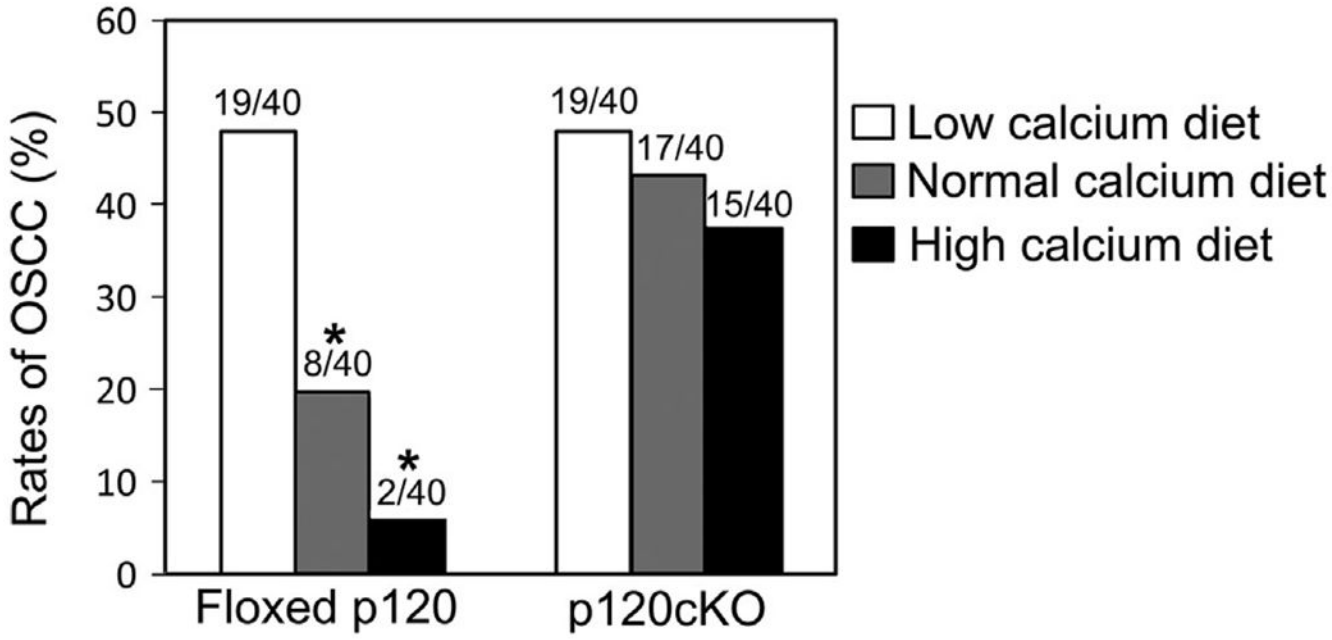

Fig. 4.

Ablation of p120 compromised calcium-suppressed OSCC formation. Floxed p120 or p120cKO mice fed the low, normal or high calcium chow diet were fed drinking water containing 4NQO (10 μg/ml) for 16 weeks. Each group had 40 mice. The mice were then fed normal drinking water for 12 weeks. At the end of 12 weeks, the mice were sacrificed, and their tongues were removed. Tumors in tongues of all mice were examined visually and every 20th sections of the tongue were stained with hematoxylin and eosin to delineate morphology. The presence of OSCC in each tongue was assessed. The number of mice with OSCC was recorded and the rates of OSCC were calculated. The Chi-square was used to determine differences among groups. Statistical significance (*$P < 0.01$) compared to the floxed p120 mice fed the low calcium diet is shown.

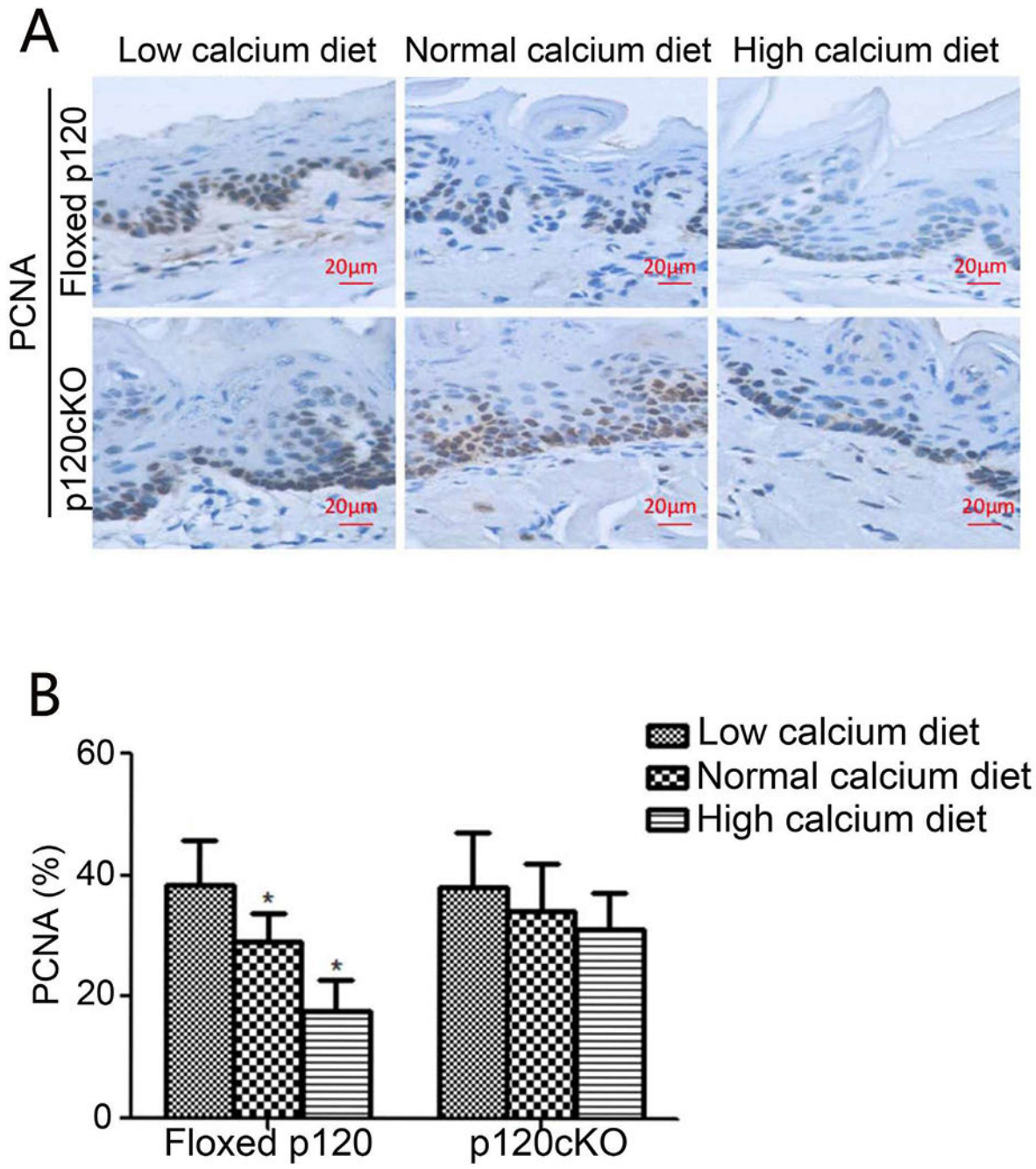

Fig. 5.

Ablation of p120 compromised calcium-suppressed proliferation of oral epithelium. Forty floxed p120 mice or p120cKO mice fed the low, normal, or high calcium diet in each group were treated with 4NQO as described in the legend for Figure 2. All mice were sacrificed and tongues were removed. Tongue tissue sections were stained with antibodies against proliferation markers PCNA. The staining on a representative section is shown (a). Quantitation of the staining is presented as a bar graph (b). Each bar represents the mean and standard deviation of the results from 10 fields. The experiment was repeated on six different

sections. One-way ANOVA was used to determine differences among groups. Comparison between the groups was performed using S–N–K method. Statistical significance (*$P < 0.05$) compared to the floxed p120 mice fed the low calcium diet is shown.

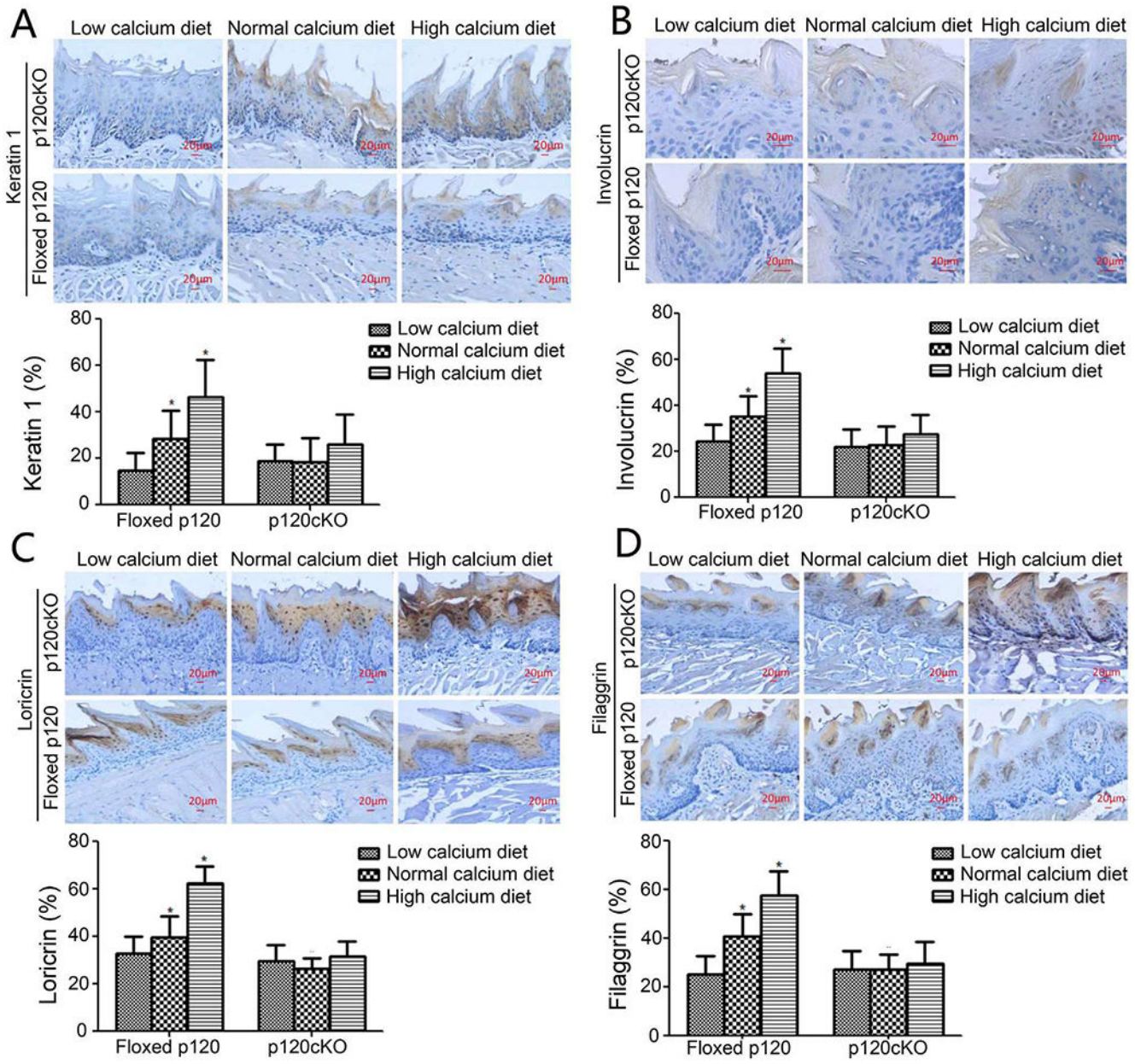

Fig. 6.

Ablation of p120 compromised dietary calcium-promoted differentiation of oral epithelium. Forty floxed p120 mice or p120cKO mice fed the low, normal, or high calcium diet in each group were treated with 4NQO as described in the legend for Figure 2. All mice were sacrificed and tongues were removed. Tongue tissue sections were stained with antibodies against epithelial differentiation markers including keratin 1, involucrin, loricrin, and filaggrin. The staining on representative sections is shown. Quantitation of the staining is presented as bar graphs. Each bar represents the mean and standard deviation of the results from 10 fields. The experiment was repeated on six different sections. One-way ANOVA was used to determine differences among groups. Comparison between the groups was

performed using S–N–K method. Statistical significance (*$P < 0.05$) compared to the floxed p120 mice fed the low calcium diet is shown.

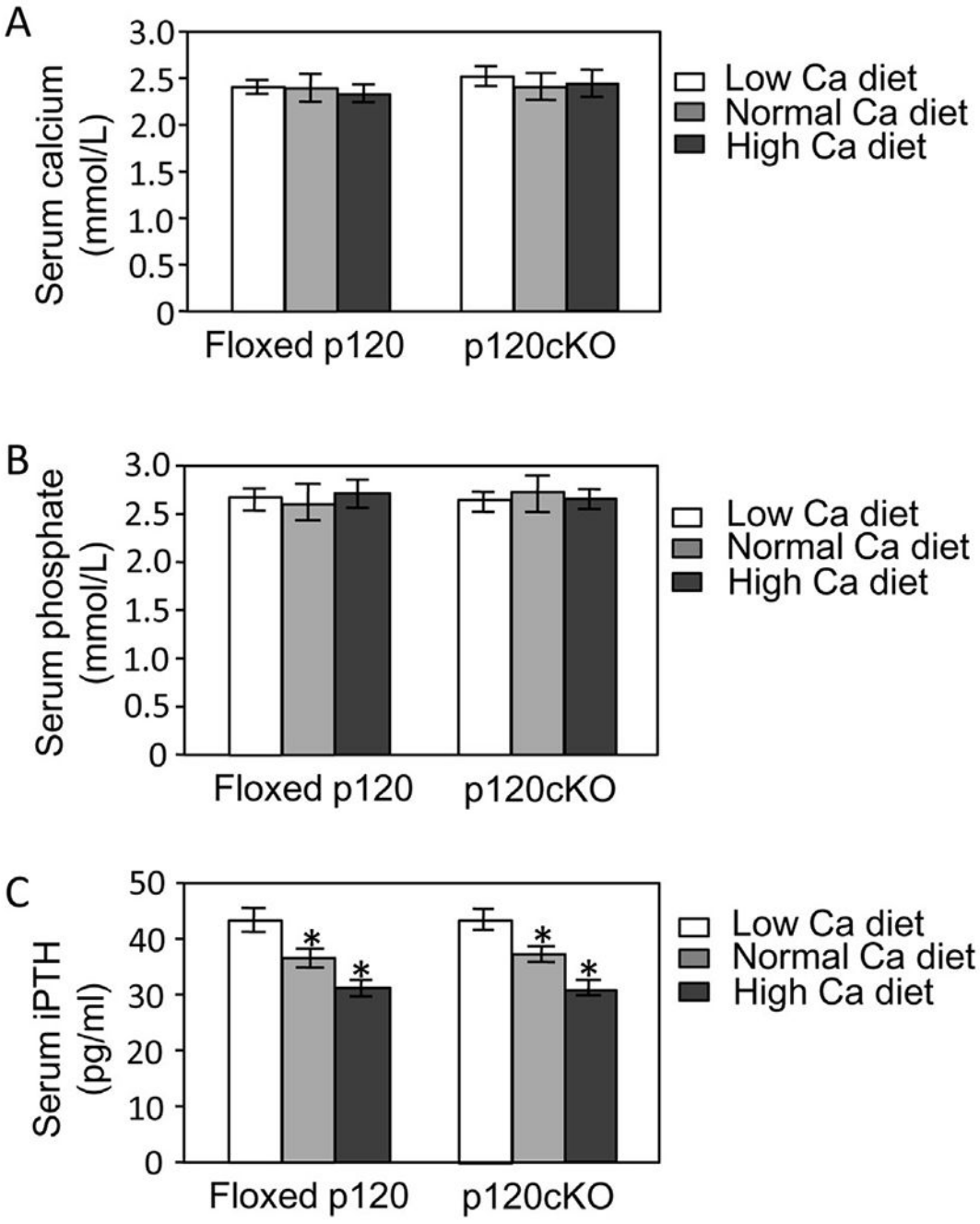

Fig. 7.

Effects of dietary calcium and ablation of p120 on calcium, phosphate, and iPTH levels in serum. Forty floxed p120 mice or p120cKO mice fed the low, normal, or high calcium diet in each group were treated with 4NQO as described in the legend for Figure 2. Levels of calcium, phosphate, and iPTH in serum from each mice in each group were measured. Each bar represents the mean and standard deviation of the results from 40 samples. One-way ANOVA was used to determine differences among groups. Comparison between the groups

was performed using S–N–K method. Statistical significance (*$P < 0.05$) compared to the floxed p120 mice fed the low calcium diet is shown.